AD NUMBER

ADB011150

LIMITATION CHANGES

TO:

Approved for public release; distribution is unlimited.

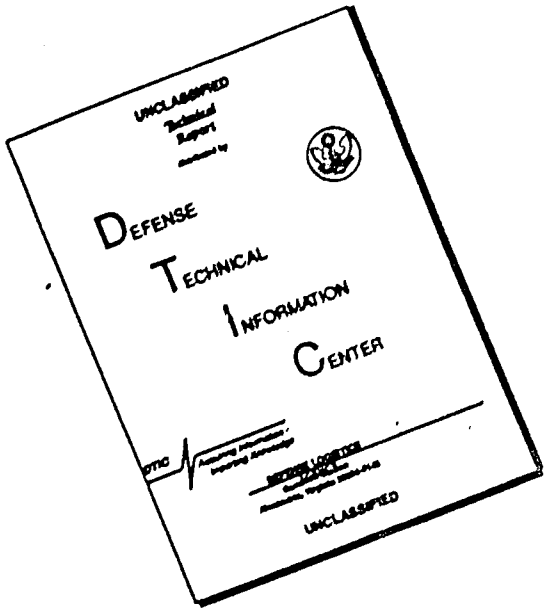
FROM:

Distribution authorized to U.S. Gov't. agencies only; Proprietary Information; 22 JUL 1975. Other requests shall be referred to U.S. Army Medical Research and Development Command, Attn: SGRD-RP, Washington, DC 20314.

AUTHORITY

MRI notice dtd 18 Mar 1977

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

MIDWEST RESEARCH INSTITUTE

REPORT

MAMMALIAN TOXICITY OF MUNITION COMPOUNDS: PHASE I. ACUTE ORAL TOXICITY PRIMARY SKIN AND EYE IRRITATION, DERMAL SENSITIZATION, AND DISPOSITION AND METABOLISM

REPORT NO. 1
22 July 1975

Contract No. DAMD-17-74-C-4073
MRI Project No. 3900-B

For

Environmental Protection Research Division
U.S. Army Medical Research and
Development Command
Washington, D.C. 20314

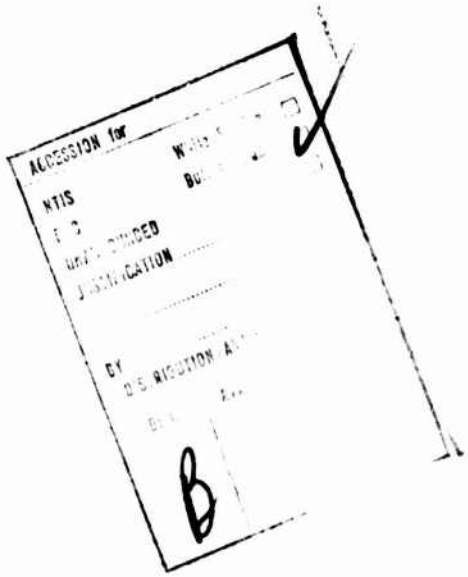
MIDWEST RESEARCH INSTITUTE 425 VOLKER BOULEVARD, KANSAS CITY, MISSOURI 64110 • 816 561-0202

Animal experimentation: Animal experiments were conducted according to the "Guide for Laboratory Animal Facilities and Care" (1965) prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council; the regulations and standards prepared by the Department of Agriculture; and Public Law 89-544, "Laboratory Animal Welfare Act," 24 August 1967.

Release: The information in this report has not been cleared for release to the general public.

Disclaimer: The findings in this report are not to be construed as an official position of the Department of the Army, unless so designated by other authorized documents.

All distribution of this report is controlled. Qualified DDC users should request through the Commanding Officer, U.S. Army Medical Research and Development Command.



(6) MAMMALIAN TOXICITY OF MUNITION COMPOUNDS. PHASE I. ACUTE ORAL TOXICITY PRIMARY SKIN AND EYE IRRITATION, DERMAL SENSITIZATION, AND DISPOSITION AND METABOLISM.

by

(10) Cheng-Chun Lee,
James V. Dilley,
John R. Hodgson,
Danny O. Helton
Wesley J. Wiegand
Dick N. Roberts
Bruce S. Andersen
Laurel M. Halfpap
Lorren D. Kurtz
Nita West

(12) 110p.

(11) REPORT NO. 1
22 July 1975

(15) Contract No. DAMD17-74-C-4073

(16) MRI [redacted]-3900-B

(9) Formal rept. no. 1, 1 Apr 74-31 May 75

For

Environmental Protection Research Division
U.S. Army Medical Research and
Development Command
Washington, D.C. 20314

230 350

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Formal Report No. 1	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Mammalian Toxicity of Munition Compounds: Phase I Acute Oral Toxicity, Primary Skin and Eye Irritation, Dermal Sensitization and Disposition and Metabolism.		5. TYPE OF REPORT & PERIOD COVERED Formal Report April 1, 1974 to May 31, 1975 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Cheng-Chun Lee, James V. Dilley, John R. Hodgson, Danny O. Helton, Wesley J. Wiegand, Dick N. Roberts Bruce S. Anderson, Laurel M. Halfpap, Loren D. Kurtz and Nita West		8. CONTRACT OR GRANT NUMBER(s) DAMD-17-74-C-4073
9. PERFORMING ORGANIZATION NAME AND ADDRESS Midwest Research Institute 425 Volker Boulevard Kansas City, Missouri 64110		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS		12. REPORT DATE July 22, 1975 13. NUMBER OF PAGES 101
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) Headquarters, U.S. Army Medical Research and Development Command Washington, D.C. 20314		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Distribution limited to U.S. Government agencies only; proprietary information; July 22, 1975. Other request for this document must be referred to the Commander, U.S. Army Medical Research and Development Command (Attn: SGRD-RP) Washington, D.C. 20314

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Toxicity	Trinitrotoluene	Dermal Sensitization	Nitrocellulose
Oral Toxicity	Trinitroglycerin	Disposition	White Phosphorus
Skin Irritation	Dinitroglycerins	Metabolism	
Eye Irritation	Mononitroglycerins		

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified

PREFACE

This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110, under U.S. Department of the Army Contract No. DAMD-17-74-C-4073, MRI Project No. 3900-B, "Munition Compounds Mammalian Toxicity Study." The work was supported by the Environmental Protection Research Division, U.S. Army Medical Research and Development Command, Department of the Army. Captain John P. Glennon, Sanitary Engineering Research Branch, is the contract officer for the project.

This work was conducted in the Biological Sciences Division, under the direction of Dr. William B. House, between 1 April 1974 and 31 May 1975. The experimental work was supervised directly by Dr. Cheng-Chun Lee, Head, Pharmacology and Toxicology; assisted by Dr. James V. Dilley, Senior Toxicologist, Dr. John R. Hodgson, Associate Biochemist, Dr. Danny O. Helton, Associate Chemist, and Mr. Wesley J. Wiegand, Senior Chemist; with the technical assistance of Dick N. Roberts, Bruce S. Andersen, Laurel M. Halfpap, Lorren D. Kurtz, and Nita West.

Approved for:

MIDWEST RESEARCH INSTITUTE

WB House/cc

W. B. House, Director
Biological Sciences Division

22 July 1975

TABLE OF CONTENTS

TABLE OF CONTENTS (Concluded)

MAMMALIAN TOXICITY OF MUNITION COMPOUNDS: PHASE I. ACUTE ORAL TOXICITY PRIMARY SKIN AND EYE IRRITATION, DERMAL SENSITIZATION, AND DISPOSITION AND METABOLISM

(Report Number 1)

ABSTRACT

Relatively pure trinitrotoluene (TNT) and its dinitroisomers (2,3-DNT, 2,4-DNT, 2,5-DNT, 2,6-DNT, and 3,4-DNT), trinitroglycerin (TNG) and its dinitroisomers (1,2-DNT and 1,3-DNT), and mononitroisomers (1-MNG and 2-MNG), nitrocellulose (NC), and white phosphorus (WP), were obtained commercially or synthesized for acute oral toxicity, primary skin and eye irritation, and dermal sensitization studies. ^{14}C-labelled nitrotoluenes and nitroglycerins and ^{32}P were used for the study of disposition and metabolism.

Acute oral LD_{50}s of the various compounds were studied in rats and mice of both sexes. NC was practically nontoxic, WP was highly toxic, and the other compounds were moderately toxic. The rats were generally more sensitive than the mice. There were no obvious sex differences. Among the nitrotoluenes, 2,4-DNT, 2,5-DNT, and 2,6-DNT were more toxic than TNT in rats; 2,5-DNT, 2,6-DNT, and 3,4-DNT were more toxic, and 2,3-DNT and 2,4-DNT were slightly less toxic than TNT in mice. Among the nitroglycerins, 1-MNG was more toxic, and both DNGs were less toxic than TNG in rats; 1,3-DNG was more toxic, and 1-MNG was less toxic than TNG in mice. 2-MNG was practically nontoxic to both rats and mice.

Rats and mice receiving toxic doses of nitrotoluenes and nitroglycerins exhibited ataxia, respiratory depression, and cyanosis. TNT and 3,4-DNT also caused coordinated and symmetrical convulsions. Death occurred within 24 hours with the nitrotoluenes, TNG, and 1,2-DNG; and between 3 and 6 days for 1,3-DNG and 1-MNG. WP caused depression, anorexia, and death in several days with enlarged yellow nutmeg livers.

2,5-DNT was moderately irritating to the rabbit skin; and TNT, the other DNTs, and TNG mildly irritating. TNT and TNG were moderate sensitizing agents in guinea pigs; and 2,6-DNT, a mild sensitizing agent. Tests for the other compounds were negative.

The nitrotoluenes (ring-UL-^{14}C), were well absorbed after oral administration in rats. The liver and kidneys contained small amounts of radioactivity. These compounds were extensively metabolized and excreted in the urine. The urinary metabolites with intact aromatic rings consisted of one or two classes of polar components.

The nitroglycerins (NG-1,3-^{14}C), were also well absorbed after oral administration in rats. The extent of absorption was slightly less for 2-MNG, correlating with its less oral toxicity. The liver contained relatively large amounts of radioactivity. These compounds were extensively metabolized and excreted in the expired air as $^{14}CO_2$ and in the urine. The urinary metabolites for TNG and the DNGs consisted of free MNGs, glycerol, glucuronides, and other polar components indicating denitration and conjugation. The urinary metabolites for MNGs consisted of glycerol and some polar components in addition to unchanged parent compounds.

The WP (^{32}P), was moderately absorbed after oral administration in rats, concentrated in the liver, and excreted in the urine. Both the urinary metabolites and the liver extract consisted of two classes of compounds. One class corresponded to inorganic phosphate. The other class was more nonpolar, suggesting an organic phosphate.

We have studied 3,5-DNT and 4-amino-2,6-DNT. We are obtaining some more material to complete the various studies. We have also obtained one batch of ^{14}C-labelled cellulose and some more material will be made during the current cotton growing season. Enough ^{14}C-cellulose will then be nitrated to produce ^{14}C-nitrocellulose for absorption and disposition experiments. Results will be summarized upon completion of these studies.

Program Director

Cheng-Chun Lee

Cheng-Chun Lee, Ph.D.
Head, Pharmacology and Toxicology
MIDWEST RESEARCH INSTITUTE
425 Volker Boulevard
Kansas City, Missouri 64110

I. INTRODUCTION

Under Contract No. DAMD-17-74-C-4073, entitled "Munition Compounds Mammalian Toxicity Study," we have completed various studies in Phase I. These studies include the acute oral toxicity of 2,4,6-trinitrotoluene (TNT), 2,3-dinitrotoluene (2,3-DNT), 2,4-dinitrotoluene (2,4-DNT), 2,5-dinitrotoluene (2,5-DNT), 2,6-dinitrotoluene (2,6-DNT), 3,4-dinitrotoluene (3,4-DNT), trinitroglycerin (TNG), 1,2-dinitroglycerin (1,2-DNG), 1,3-dinitroglycerin (1,3-DNG), 1-mononitroglycerin (1-MNG), 2-mononitroglycerin (2-MNG), nitrocellulose, and white phosphorus in rats and mice; the primary skin and eye irritation in rabbits; the dermal sensitivity in guinea pigs; and the absorption and disposition of ^{14}C-labeled compounds (except ^{14}C-labeled nitrocellulose) after oral administration in rats. In addition, some preliminary information was obtained on the general classification of metabolites of ^{14}C-labeled compounds found in the urine of treated rats. For these studies, the various compounds, including the radiochemicals (except nitrocellulose), were procured or synthesized. Their identity and purity were determined. The particle size and nitrogen content analyses were performed on poacher pit samples of nitrocellulose. This report summarizes these results.

The various studies on 3,5-dinitrotoluene (3,5-DNT) and on 4-amino-2,6-dinitrotoluene (4-amino-2,6-DNT) have also been performed. We are continuing the acute oral toxicity of 3,5-DNT in mice and are waiting for some more 4-amino-2,6-DNT for the acute oral toxicity in both rats and mice and dermal sensitivity tests in rabbits. One batch of 14.25 µCi of ^{14}C-labeled cellulose was obtained by growing cotton plants in synthetic medium containing ^{14}C-glucose. An additional batch will be prepared during the growing season in August. When a sufficient amount of ^{14}C-labeled cellulose is obtained, it will be nitrated to produce the ^{14}C-labeled nitrocellulose for the absorption and disposition study. After completion of these experiments, a complete report on the various studies, including the acute oral toxicity in rats and mice, primary skin and eye irritation tests in rabbits, dermal sensitivity tests in guinea pigs, absorption and disposition in rats of 3,5-DNT and 4-amino-2,6-DNT, and on the absorption and disposition of nitrocellulose will be prepared and submitted.

II. MATERIALS AND METHODS

A. Animals

Male and female Charles River CD® rats were obtained from the Charles River Breeding Laboratories (North Wilmington, Massachusetts). Male and female albino Swiss mice were obtained from the National Laboratory

Animal Company (O'Fallon, Missouri). Guinea pigs and New Zealand rabbits were obtained from Small Stock Industries (Pea Ridge, Arkansas).

All animals were kept in air conditioned rooms (75 ± 5°F) with relative humidity of 50 ± 10%, and photoperiod of 12 hours. They were supplied with Purina rodent chow and water ad libitum. All animals were kept under observation for 1 week after arrival to insure that only healthy animals were used. Rats, mice, and guinea pigs were housed in plastic cages and provided with hardwood bedding; rabbits were housed in metal cages with wire bottoms.

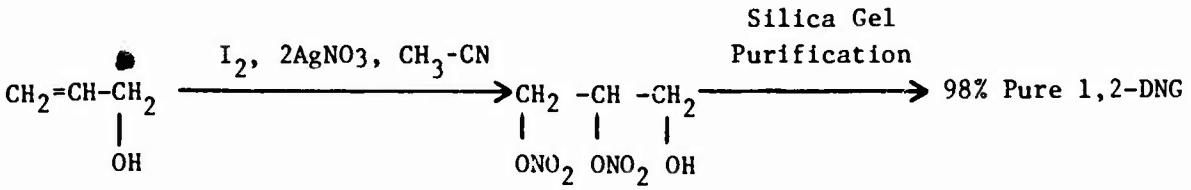
B. Chemicals

1. Sources of TNT, DNTs, TNG, Nitrocellulose, and White Phosphorus

TNT and all the DNT isomers were purchased from K&K Laboratories (Cleveland, Ohio). TNG was obtained as a 10% mixture in lactose from ICI America--Atlas Chemical Division (Chicago, Illinois). White phosphorus was obtained from J. T. Baker Chemical Company (Phillipsburg, Pennsylvania). Nitrocellulose was supplied by the Radford Army Ammunition Plant (Radford, Virginia).

2. Synthesis of DNGs and MNGs

a. 1,2-DNG: The sample was prepared by a method similar to that of Dunstan et al.[1/] as shown in the diagram.

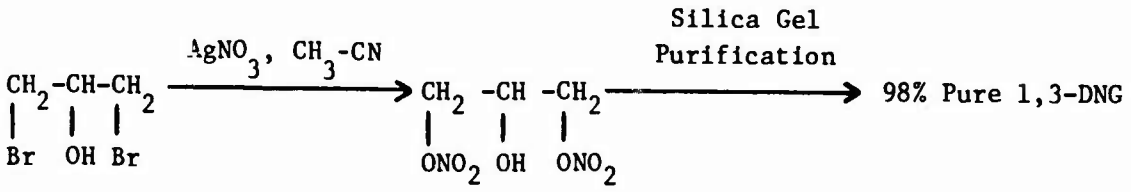


To a solution of 11.6 gm (0.2 mole) of allyl alcohol and 67.9 gm (0.4 mole) of silver nitrate dissolved in 300 ml acetonitrile was added 25.4 gm (0.2 mole) of iodine dissolved in 500 ml of acetonitrile during a 2.5-hour period. After 2 hours, the mixture was placed in a water bath at 50°C for 24 hours. The mixture was filtered to remove the silver iodide and concentrated to between 100 and 200 ml. The filtrate was again placed in a water bath at 50°C. After 3 days, additional

silver nitrate (5 gm) was added. After 7 days, the reaction mixture was filtered. To remove excess silver nitrate, the filtrate was poured into 100 ml of water saturated with sodium chloride; the mixture was filtered to remove silver chloride. Acetonitrile was removed under water vacuum (about 20 mm Hg). The water solution was extracted three times with 60-ml portions of ether. The ether extracts were combined, dried over anhydrous magnesium sulfate, diluted with 75 ml hexane/ethyl acetate (2:1) and the volume reduced to about 50 ml under water vacuum. The approximate concentration of material per ml was determined by evaporating the solvent from a 1.0-ml aliquot and weighing.

Purified 1,2-DNG was obtained by column chromatography on silica gel (40:1 ratio of silica gel to material) using hexane/ethyl acetate (2:1) as eluant. The fractions (100 ml volume) were monitored by thin-layer chromatography (Brinkmann silica gel F-254, hexane/ethyl acetate (2:1)). Two column chromatographs were required to give pure material (> 98%). Six preparations gave an average of 6.9 gm (24% yield) per preparation. The purified material was stored at a 20-30% concentration in hexane/ethyl acetate (2:1), protected from light at 3-5°C.

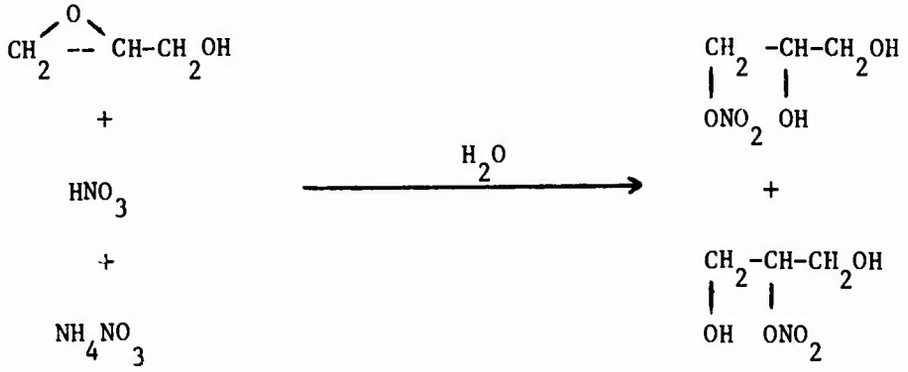
b. 1,3-DNT: The sample was also prepared by a method similar to that of Dunstan et al.[1] as shown in the diagram.



To a solution of 34.0 gm (0.2 mole) of silver nitrate in 100 ml of acetonitrile was added dropwise 21.8 gm (0.1 mole) of 1,3-dibromo-2-propanol. After 2 days at 50°C, 5 gm of silver nitrate dissolved in 50 ml of acetonitrile was added. After 7 days at 50°C, the solution was filtered and the filtrate poured into 100 ml of water saturated with sodium chloride to remove excess silver nitrate. The solution was filtered to remove silver chloride followed by removal of acetonitrile under water vacuum. The water solution was extracted four times with 150-ml portions of ethyl ether. The combined ether solutions were dried over sodium sulfate, filtered, and concentrated to about 30 ml. This solution was divided into two equal volumes and each chromatographed on 240 gm of silica gel (Brinkmann, 70-230 mesh) using hexane/ethyl acetate (2:1). The fractions (100 ml volume) were monitored by thin-layer chromatography (Brinkmann silica gel F-254, hexane/ethyl

acetate (2:1)). After two column chromatographs per portion, 15 gm (82% yield) of > 98% pure material was obtained. The purified material was stored at a 20-30% concentration in hexane/ethyl acetate (2:1), protected from light at 3-5°C.

c. MNGs: Both 1-MNG and 2-MNG were prepared in the same reaction. The method is similar to that of Nichols et al.[2] as shown in the diagram.



To a 60°C solution containing 40 ml of H_2O, 85 ml of 70% HNO_3, and 250 gm of NH_4NO_3 was added dropwise 100 gm of glycidol over a 25-minute period. After an additional 15 minutes, the solution was neutralized with $NaHCO_3$ (powder) until the evolution of CO_2 ceased.

An extra 100 ml of H_2O was added, followed by extraction with 250 ml of ethyl ether to remove DNGs. The ether solution was discarded. The aqueous solution was extracted with ethyl ether using a continous liquid-liquid extractor for 24 hours. The ether extract contained 80-120 gm (30-50% yield) of 1- and 2-mononitroglycerin in a 3:1 ratio, respectively.

The ether solution was dried over sodium sulfate and diluted to 500 ml. After removal of the sodium sulfate, 200 gm of $CaCl_2$ was added slowly with vigorous stirring. After standing overnight, the solution was decanted and the precipitate was washed with three 100-ml portions of ether. The combined ether solutions contained 80-90% 2-MNG. The $CaCl_2$ complex contained about 90% 1-MNG.

The 2-MNG was further purified by passing 50 gm batches through a 2 in. x 24 in. liquid chromatography column packed with $CaCl_2$ in ether. This gave a high purity of 2-MNG. The material recrystallized from benzene/ether (1:1) to give small needles.

The 1-MNG was recovered by adding water to the $CaCl_2$ complex and continuous extraction with ethyl ether. Repeating the complexing and recovery procedure gave material which, after two recrystallizations, was 99% 1-MNG. The material recrystallized readily from benzene/ether (1:1).

3. Analysis of Chemicals

a. Nitrotoluenes and nitroglycerins: The identity of various lots of nitrotoluenes and nitroglycerins was determined by a combination of the following techniques: melting point, infrared spectrum, ultraviolet spectrum, and nuclear magnetic resonance. Their assay was determined by a combination of the following techniques: elemental analysis, thin-layer chromatography, and gas chromatography.

b. Nitrocellulose: The nitrocellulose was assayed for its nitrogen content according to the method of Selig.[3] It was also analyzed for particle size by sieving and examined by microscopy.

C. Acute LD_{50}

Rats and mice were fasted for at least 16 hours prior to oral dosing. Oral doses were administered to rats and mice by intragastric intubation via a stainless steel tube. After treatment, the survivors were observed daily for 14 days for delayed mortality or toxic signs. The LD_{50} was calculated for each compound by a computer program based on the method of maximum likelihood of Finney.[4]

The solutions of TNT, 2,4-DNT, and 2,6-DNT for toxicity testing were prepared by saturating peanut oil with the compound and then chemically assaying for the final concentration. The other DNTs were prepared by dissolving weighed amounts of the compound in peanut oil. The TNG in lactose (9.72%) was added to peanut oil to make a 35:65 mixture. The final solution of TNG was found to contain 3.41% TNG, 31.5% suspended lactose, and 65% peanut oil. A 35% suspension of lactose in peanut oil was then used as a vehicle control. The DNGs were prepared by adding known amounts to peanut oil. The MNGs are very soluble in water. Equal parts of MNGs and water (weight/volume) were mixed. The resulting solutions were emulsified in the peanut oil so that the final concentration of the emulsion was 10% MNGs. Nitrocellulose was ground in a blender and the resultant material was suspended in water at a final concentration of 5% nitrocellulose (dry weight basis). White phosphorus was dissolved directly in peanut oil as a 1.0% solution. This was diluted to a 0.1% solution in peanut oil for use.

D. Primary Skin and Eye Irritation

Primary skin and eye irritation tests were carried out according to the modified Draize method as described in the Federal Register.[5] Rabbits were clipped free of hair over the appropriate skin areas at least 24 hours prior to testing. After application of the test material, the irritation score on intact and abraided skin and the eye was evaluated at 24 and 72 hours.

The nitrotoluenes were prepared as a 50% paste with peanut oil just prior to application. TNG was prepared as a paste which contained 25% peanut oil and 75% of the TNG (9.72%) in lactose. This paste was calculated to contain 7.29% TNG and 67.21% lactose. A 67% suspension of lactose in peanut oil was used for vehicle control. The DNGs were applied as 10% solutions in peanut oil and the MNG as 10% emulsions in peanut oil and water. Nitrocellulose concentration was 33% in water. White phosphorus was applied as a 0.1% solution in peanut oil.

E. Dermal Sensitivity Studies

Dermal sensitivity test was performed in guinea pigs according to the "maximization test" described by Magnusson and Kligman.[6] Test animals were clipped free of hair at least 24 hours prior to testing or prior to the final challenge with the test substances. The preparations of the nitrotoluenes and TNG used for the dermal sensitization tests were the same as those used for acute oral toxicity study.

F. Disposition and Metabolism

1. Radiochemicals

The ^{14}C-labeled nitrotoluenes (ring-UL-^{14}C) and nitroglycerins (NG-1,3-^{14}C) were prepared by the New England Nuclear Corporation (Boston, Massachusetts). Their specific activities are listed in Table 1. The ^{32}P-white phosphorus was also prepared by New England Nuclear Corporation according to the procedure outlined below:

*Red phosphorus was neutron activated to produce ^{32}P-labeled red phosphorus.

*^{32}P-labeled red phosphorus was sublimed at 400°C to produce ^{32}P-labeled white phosphorus.

*^{32}P-labeled white phosphorus was dissolved in CS_2 and filtered through glass wool into a shipping vial.

*The vial was taken to dryness with N_2.

*The ^{32}P-labeled white phosphorus was resuspended in peanut oil and shipped to MRI.

2. Experimental Procedure

Charles River CD® female rats weighing between 175 and 250 gm were used. Each rat was fasted overnight before being given a single oral dose of approximately 1/10 of the LD_{50} of the test compound, spiked with 10 µCi of the ^{14}C-labeled compound. The test material was suspended in peanut oil and given via an intragastric tube at a volume of 1 ml/100 gm body weight. After dosing, each rat was placed immediately in a "Roth-Delmar" metabolism cage[7] with food and water ad libitum. The chamber was vented continuously with CO_2-free air at a rate of 250 ml/min. Expired CO_2 was collected by bubbling the air through six absorption columns connected in series. Each column contained 100 ml of 5% NaOH. Feces and urine were collected separately in the apparatus. At the termination of each experiment, the rat was anesthetized with ether and aortic blood collected in a heparinized syringe. Liver, kidneys, brain, lungs, and thigh muscle were removed, weighed, and representative samples taken for analysis of radioactivity. The gastrointestinal tract plus contents (GI) was removed and weighed. The GI and the feces were homogenized in three volumes of dioxane[8] (or water in the case of white phosphorus) and filtered; samples of the filtrate and the filtered residue were assayed for radioactivity.

3. Radioactive Assays

Aliquots of whole blood, tissue samples, and filtrate residues were digested in 2N NaOH. The carcasses were digested in 10 volumes 6N NaOH. Blood samples were decolorized by dropwise addition of hydrogen peroxide. Samples of tissue digests were neutralized with Beckman BBS-2, solubilized in Beckman BBS-3, and counted in a toluene-PPO-dimethyl POPOP cocktail using a Packard Tricarb 3375 liquid scintillation spectrometer. Samples of plasma, urine, dioxane filtrates, and test radiochemicals were solubilized directly in BBS-3 and counted. $^{14}CO_2$ samples from the air traps were spotted on filter paper, dried, and counted. All data were corrected for background and quenching.

4. Thin-Layer Chromatography (TLC)

Precoated Silica Gel 60 plates (without fluorescent indicator, 0.25 mm thickness, E. M. Laboratories, Inc., Elmsford, New York) were used for all experiments. All samples were spotted 2.0 cm from the bottom of the plate and developed for a minimum of 10 cm. The solvents

used were: (a) benzene:ethyl acetate (4:1, v/v); (b) ethyl acetate:n-heptane (9:1, v/v); (c) n-butanol:acetic acid:water (5:1:4, v/v/v); (d) n-butanol:methanol:water (120:33:57, v/v/v); (e) petroleum ether:ethyl acetate (15:85); and (f) 1,2-dichloroethane:petroleum ether (25:75). Solvents (c) and (d) were used with chamber saturation. Solvent systems (a) through (d) were used for the identification of nitroglycerins and their metabolites; solvent systems (d) through (f), for nitrotoluenes and their metabolites.

5. Gas-Liquid Chromatography (GLC)

A Bendix-2500 gas chromatograph equipped with an electron capture detector was used for GLC on stainless steel columns (0.125 in. inside diameter x 6 ft). The column was packed with 1.5% DC LSX-3-2095 and 1.5% GE-XE-60 on Gas Chrom Q. The column and detector temperatures were 120°C and 220°C, respectively. Nitrogen carrier gas was used at a flow rate of 120 ml/min. Samples for GLC analysis were collected by eluting the metabolites from TLC scrapings with water.

6. Chemical Detection Tests

Nitrotoluenes and nitroglycerins were detected using 5% diphenylamine spray reagent[9] followed by UV-irradiation. Glycerol was detected using the alkaline-$KMnO_4$ spray reagent.[10]

7. β-Glucuronidase Treatment

Samples were prepared for enzyme treatment by eluting the metabolites from TLC scrapings with water. The pH was adjusted to 5.6 by the addition of 13.6 mg/ml of sodium acetate. β-Glucuronidase (Sigma Chemical Company, St. Louis, Missouri) was added at a concentration of 5.0 mg/ml and the solution incubated at 37°C for 18 hours. After incubation, enzyme activity was terminated by extraction with 5 volumes of $CHCl_3$:MeOH (2:1). The resulting aqueous and nonaqueous phases were concentrated by evaporation and the metabolites identified by TLC.

8. Tissue Extraction

Tissue extracts were prepared by homogenizing the tissue in water and subsequent extraction with 5 volumes of $CHCl_3$:MeOH (2:1). The resulting aqueous and nonaqueous phases were concentrated by evaporation and the metabolites characterized by TLC.

III. RESULTS

A. Chemical Analyses

The identification and assay of the nitrotoluenes and nitroglycerins are described in detail in Appendix I.

1. Nitrotoluenes

The identity of the nitrotoluenes was determined and the purity is listed below. The 2,4-DNT also contained 2% 2,6-DNT. The 2,5-DNT also contained 1% 2,3-DNT and 4% 2,6-DNT. The TNT and other DNTs were more than 99% pure.

Nitrotoluenes	Purity
TNT	> 99%
2,3-DNT	> 99%
2,4-DNT	98% 2,4-DNT, 2% 2,6-DNT
2,5-DNT	95 ± 1% 2,5-DNT, 1% 2,3-DNT, 4 ± 1% 2,6-DNT
2,6-DNT	> 99%
3,4-DNT	> 99%

2. Nitroglycerins

The identity of nitroglycerins was determined and the purity is listed below. The TNG contained 9.72% in lactose. The 2-MNG also contained 1.5% 1-MNG. The DNGs and 1-MNG were more than 99% pure.

Nitroglycerins	Purity
TNG	9.72 ± 0.09% in lactose
1,2-DNG	> 99%
1,3-DNG	> 99%
1-MNG	> 99%
2-MNG	98.5% 2-MNG, 1.5% 1-MNG

3. Nitrocellulose

The nitrogen content of nitrocellulose from Line B at Radford Army Ammunition Plant was found to be 13.1 ± 0.1%. Wet sieving indicated the following weight distribution according to particle size:

Particle Size (μ)	% Weight Distribution
> 88	65.6
< 88 but > 44	23.2
< 44	11.2

Optical microscopy indicated the > 44 μ particles to be normal nitrocellulose fibers. Those particles < 44 μ appeared in several forms, including amorphous and spherical, as well as appearing to be simply fragments of larger nitrocellulose particles. Work is continuing in this area.

B. Biological Studies

The acute oral LD_{50}s of each compound in rats and mice are listed in Tables 2 and 3, respectively. The results of the primary skin and eye irritation tests in rabbits are shown in Tables 4 and 5. The results of the dermal sensitivity tests in guinea pigs are listed in Table 6. The results of the disposition and metabolism in rats are summarized in Tables 7 through 27. The characterization of metabolites are illustrated in Figures 1 through 9.

1. TNT

TNT was administered as a 4.12% solution in peanut oil. The acute LD_{50} ± S.E. (95% confidence limits) in male and female rats were 1,010 ± 41 (922-1,108) and 820 ± 32 (747-889) mg/kg, respectively; in male and female mice were 1,014 ± 52 (905-1,163) and 1,009 ± 54 (880-1,117) mg/kg, respectively. Within 5 to 15 minutes after dosing, the animals exhibited symmetrical coordinated convulsions. The convulsions continued for 1 to 2 hours. If the animals survived the respiratory inhibition associated with the convulsions, they survived the dose. The survivors appeared cyanotic and exhibited ataxia after the convulsions but recovered completely in 24 to 48 hours. No gross pathology attributable to the treatment was noted in animals that died. A bright red pigment appeared in the urine of both rats and mice within 10 to 20 minutes after dosing. This pigment stained the fur and bedding for several days after dosing.

TNT was a mild skin irritant in rabbits but did not produce eye irritation. A red pigment appeared on the skin and around the eye after the TNT was applied. It was a moderate sensitizing agent in guinea pigs.

The distribution and excretion of radioactivity in rats after an oral dose of radiolabeled TNT are summarized in Table 7. The animals killed at 30 minutes after dosing were given a lethal dose in order to determine how much TNT was getting into the brain to cause

convulsions. Only 0.1% of the administered dose was found in the brain. About 60 to 74% of an oral nonlethal dose was absorbed in 24 hours. At 24 hours after dosing, 26% of the radioactivity was found in the gastrointestinal tract and the feces. Most of the absorbed radioactivity was excreted in the urine averaging 53.3% of the administered dose. A negligible amount of radioactivity was recovered in the expired air. Small but significant amounts of radioactivity were also found in the blood, liver, kidney, and skeletal muscle. The tissue to plasma radioactivity ratio suggested some retention of radioactivity in the liver and kidney.

A TLC analysis of the radioactivity in the urine and in the brain using a solvent system of ethyl acetate:petroleum ether is shown in Figure 1. All the radioactivity in the 30-minute and 24-hour urine samples remained at the origin whereas TNT has an R_f value of about 0.75 in this system. The radioactivity from the 30-minute brain extract had an R_f of 0.2, indicating the presence of one metabolite different from that in the urine. A 24-hour urine sample was also developed in a butanol:methanol:water system. There were one major and several minor peaks of radioactivity. Practically no radioactivity was associated with the TNT or DNTs which have R_f values of about 0.95 in this solvent system.

2. 2,3-DNT

A 3% solution of 2,3-DNT in peanut oil was used for the acute toxicity studies. The acute oral LD_{50} ± S.E. (95% confidence limits) in male and female rats were 1,102 ± 20 (1,011-1,169) and 911 ± 65 (584-1,049) mg/kg, respectively; in male and female mice were 1,372 ± 34 (1,285-1,441) and 1,089 ± 32 (1,029-1,175) mg/kg, respectively. All animals exhibited cyanosis and ataxia after dosing. Death occurred within 24 hours of dosing or not at all. The survivors recovered from all toxic signs within 48 hours. No gross pathology attributable to the treatment was noted in the animals that died.

The primary skin irritation test in rabbits indicated that 2,3-DNT was a mild skin irritant. Primary eye irritation test in rabbits was negative. No dermal sensitivity was observed in guinea pigs after treatment with 2,3-DNT.

The distribution and excretion of radioactivity in rats after an oral dose of radiolabeled 2,3-DNT are summarized in Table 8. About 60% of the administered dose was absorbed in 24 hours. The radioactivity recovered in the gastrointestinal tract and the feces averaged 76.4% and 39.0% of the dose at the end of 4 and 24 hours, respectively. Most of the absorbed dose was accounted for in the urine. A negligible amount of radioactivity was recovered in the expired air. A small but significant amount of the administered radioactivity was found in the blood, liver,

kidneys, and skeletal muscle. The tissue to plasma radioactivity ratios indicate some retention of radioactivity in the liver and kidneys.

A TLC analysis of the radioactivity in the 24-hour urine sample using two different solvent systems is shown in Figure 2. There were one major and one minor peak of radioactivity, suggesting the presence of at least two metabolites. Unchanged 2,3-DNT was not detected in either solvent system.

3. 2,4-DNT

A 3.97% solution of 2,4-DNT in peanut oil was used for the acute toxicity studies. The acute oral LD_{50} ± S.E. (95% confidence limits) in male and female rats were 568 ± 59 (434-705) and 650 ± 49 (520-743) mg/kg, respectively; in male and female mice were 1,954 ± 68 (1,848-2,178) and 1,340 ± 67 (1,205-1,500) mg/kg, respectively. All the treated animals became cyanotic and ataxic after dosing. Death occurred usually within the first 24 hours or not at all. The surviving animals were completely recovered in 48 hours. No gross pathology attributable to the treatment was noted in the animals that died.

2,4-DNT was a very mild primary skin irritant in rabbits. No primary eye irritation was seen in rabbits; and no dermal sensitivity was noted in guinea pigs following 2,4-DNT treatment.

The distribution and excretion of radioactivity in rats after an oral dose of radiolabeled 2,4-DNT are summarized in Table 9. About 80 to 90% of the administered dose was absorbed and the absorption was essentially completed in 24 hours. The radioactivity recovered in the gastrointestinal tract and the feces averaged 60.3%, 11.9%, and 11.3% of the dose at the end of 4 hours, 1 day, and 5 days, respectively. Most of the absorbed radioactivity was excreted in the urine with an average of 29.3%, 75.9%, and 85.6% of the dose during the respective periods. A negligible amount of radioactivity was recovered in the expired air. Small but significant amounts of radioactivity were found in the blood, liver, kidneys, and skeletal muscle.

The tissue to plasma radioactivity ratios (Table 10) indicated a retention of radioactivity in various tissues. Another group of rats was treated with radiolabeled 2,4-DNT for 5 consecutive days to compare the amount of radioactivity in each tissue 24 hours after the last dose with that in the tissue 24 hours after a single dose. The results of these experiments are summarized in Table 11. All the tissues receiving 5 daily doses contained 2.0 to 4.6 times as much radioactivity as those receiving a single dose, indicating major accumulation in all tissues.

A TLC analysis of the radioactivity in the 4-hour and 24-hour urine samples using two different solvent systems is shown in Figure 3. There was one major peak of radioactivity in the 4-hour urine sample. In addition to this major peak, there was one minor peak in the 24-hour urine sample using the butanol:methanol:water solvent system. This minor peak of radioactivity had an R_f value corresponding to that of DNTs. However, unchanged 2,4-DNT was not found with the ethyl acetate: petroleum ether solvent system.

4. 2,5-DNT

A 4% solution of 2,5-DNT in peanut oil was used for the acute toxicity studies. The acute oral LD_{50} ± S.E. (95% confidence limits) for male and female rats were 616 ± 34 (532-707) and 517 ± 25 (477-575) mg/kg, respectively; for male and female mice were 652 ± 28 (585-712) and 659 ± 12 (633-690) mg/kg, respectively. Animals appeared cyanotic and ataxic after dosing. Death occurred within 24 hours or the animals recovered completely. No gross pathology attributable to the treatment was noted in the animals that died. All the animals had a bright yellow pigment in the urine after dosing.

The primary skin irritation test in rabbits indicated that 2,5-DNT produced a necrosis of the intact and abraded skin in 24 hours. However, there was a total absence of edema in and around the necrotic area. No primary eye irritation was observed in rabbits and no dermal sensitivity in guinea pigs after treatment with 2,5-DNT.

The distribution and excretion of radioactivity in rats after an oral dose of radiolabeled 2,5-DNT are summarized in Table 12. About 60 to 70% of the administered dose was absorbed in 24 hours. The radioactivity recovered in the gastrointestinal tract and the feces averaged 85.5% and 31.0% at the end of 4 and 24 hours, respectively. Most of the absorbed dose was accounted for in the urine. A negligible amount of radioactivity was recovered in the expired air. Small but significant amounts of radioactivity were found in the blood, liver, kidney, and skeletal muscle. The tissue to plasma radioactivity ratios suggested a strong retention of radioactivity in the liver and some retention in the kidneys and lungs.

A TLC analysis of the radioactivity in the 24-hour urine samples using two solvent systems is shown in Figure 4. Unchanged 2,5-DNT was not detected. With the butanol:methanol:water solvent system, two peaks of radioactivity were found, suggesting at least two radioactive compounds.

5. 2,6-DNT

A 4.99% solution of 2,6-DNT in peanut oil was used for the acute toxicity studies. The acute LD_{50} ± S.E. (95% confidence limits) in male and female rats were 535 ± 58 (397-646) and 795 ± 22 (744-844) mg/kg, respectively; in male and female mice were 621 ± 51 (488-721) and 807 ± 35 (735-893) mg/kg, respectively. Cyanosis and ataxia were noted in all animals after dosing. Death occurred within 24 hours of dosing or not at all. The survivors were completely recovered within 48-hours of dosing. No gross pathology attributable to the treatment was noted in the animals that died.

2,6-DNT was a borderline mild skin irritant in rabbits but nonirritating to the rabbit eye. The dermal sensitivity test indicated that it was a mild sensitizing agent in guinea pigs.

The distribution and excretion of radioactivity in rats after an oral dose of radiolabeled 2,6-DNT are summarized in Table 13. About 60% of the administered dose was absorbed in 24 hours. The radioactivity recovered in the gastrointestinal tract and the feces averaged 83.5% and 38.0% at the end of 4 and 24 hours, respectively. Most of the absorbed dose was accounted for in the urine. A negligible amount of radioactivity was recovered in the expired air. Small but significant amounts of radioactivity were found in the blood, liver, kidney, and skeletal muscle. The tissue to plasma radioactivity ratios indicated some retention of radioactivity in the liver, kidneys, lungs, and spleen.

A TLC analysis of radioactivity in the 24-hour urine samples using two different solvent systems is shown in Figure 5. Unchanged 2,6-DNT was not detected in the urine. With the butanol:methanol:water solvent system, one major peak and one minor peak of radioactivity were present, indicating the presence of at least two metabolites.

6. 3,4-DNT

A 3% solution of 3,4-DNT in peanut oil was used for the acute toxicity studies. The acute oral LD_{50} ± S.E. (95% confidence limits) in male and female rats were 907 ± 42 (815-1,011) and 807 ± 33 (721-874) mg/kg, respectively; in male and female mice were 859 ± 37 (787-958) and 747 ± 26 (702-821) mg/kg, respectively. The animals exhibited symmetrical and coordinated convulsions 5 to 15 minutes after dosing. The convulsive period lasted for 1 to 2 hours. If the animals recovered from the respiratory depression and/or paralysis associated with the convulsions, they usually survived the dose. Death occurred at the end of a convulsion and the animals died in rigor mortis. Cyanosis was apparent in all animals that were treated with 3,4-DNT. The survivors recovered completely in 24 to 48 hours. A bright yellow pigment appeared in the urine of all

treated animals within 10 to 15 minutes after dosing. This pigment stained the fur and bedding for several days after treatment. No gross pathology attributable to the treatment was seen in the animals.

Primary skin irritation test in rabbits indicated that 3,4-DNT was a mild skin irritant. Primary eye irritation test in rabbits was negative. A yellow pigment appeared on the skin in the treatment area and around the eye. No dermal sensitivity was found in guinea pigs after treatment with 3,4-DNT.

The distribution and excretion of radioactivity in rats after an oral dose of radiolabeled 3,4-DNT are summarized in Table 14. Some rats were examined 30 minutes after a minimum lethal dose of 3,4-DNT in order to determine the amount of radioactivity getting into the brain after a convulsive dose. Less than 0.1% of the administered dose was found in the brain. After an oral nonlethal dose, about 85 to 90% was absorbed in 24 hours. The radioactivity recovered in the gastrointestinal tract and the feces averaged 14.7% at the end of 24 hours. The absorbed dose was nearly accounted for in the urine. A negligible amount of radioactivity was recovered in the expired air. Less than 0.1% of the dose was found in the blood, liver, kidney, brain, and lungs. However, the tissue to plasma radioactivity ratios suggested some retention of radioactivity in the liver and kidneys.

A TLC analysis of radioactivity in the 24-hour urine sample using two solvent systems is shown in Figure 6. Only one major peak of radioactivity was detected in each solvent system and neither one corresponded to the parent compound.

A brain homogenate was extracted with water and with chloroform: methanol to give aqueous and lipid fractions. A TLC analysis of these fractions using two solvent systems is shown in Figure 9. The major peak of radioactivity detected from the aqueous extract in both solvent systems corresponded to the parent compound. The major peak detected from the lipid fraction did not correspond to 3,4-DNT.

7. TNG

The 9.7% TNG in lactose was added to peanut oil to give a final TNG concentration of 3.41% and used for the acute toxicity studies. The acute oral LD_{50} ± S.E. (95% confidence limits) in male and female rats were 822 ± 54 (700-953) and 884 ± 61 (763-1,055) mg/kg, respectively; in male and female mice were 1,188 ± 76 (1,008-1,352) and 1,055 ± 63 (895-1,178) mg/kg, respectively. Within 1 hour after dosing, all the animals became cyanotic and ataxic. The ears, nose, eyes, paws, and tail appeared very pale and the respiration was depressed. Death usually occurred

within 5 to 6 hours of dosing. The animals that survived the dose usually recovered in 24 hours. No gross pathology was noted in the animals that died.

TNG was a very mild skin irritant. Primary irritation test in rabbits indicated that TNG was not an eye irritant. The dermal sensitivity test in guinea pigs elicited a 40% positive response with TNG. This indicates that TNG is a moderate sensitizing agent.

The distribution and excretion of radioactivity in TNG-1,3-^{14}C treated rats are summarized in Table 15. About 80 to 90% of an oral dose was absorbed in 24 hours. The radioactivity remaining in the gastrointestinal tract averaged 59.5% and 3.0% at the end of 4 and 24 hours, respectively. The amounts of radioactivity in the feces during the same time periods were 3.1% and 6.3%. Most of the absorbed radioactivity was excreted in the urine and expired air, averaging 39.8% and 25.5% of the administered dose at the end of 24 hours, respectively. A significant amount of radioactivity was found in the liver, averaging 4.6% of the dose at 4 hours; the amount of radioactivity remained at this level at 24 hours. Small but significant amounts of radioactivity were also found in the various other tissues. The tissue to plasma radioactivity ratios suggested that the liver and kidneys retained the TNG and/or its metabolites. Although the muscle radioactivity was rather high at 4 hours (9.3% of the administered radioactivity), the concentration declined to 2.8% in 24 hours in direct proportion to the decline in plasma concentration.

TLC analysis of radioactivity in the 24-hour urine from TNG-1,3-^{14}C treated rats is summarized in Table 16. Only a trace of the unchanged TNG was found. An average of 0.7% and 0.4% of the administered radioactivity were free 1,2-DNG and 1,3-DNG, respectively. The free MNGs accounted for 10.6% of the administered radioactivity. TLC analysis of the MNGs indicated the ratio of 1-MNG/2-MNG to be 0.9 ± 0.2. The majority of the radioactivity was unidentified polar components. These polar components from the urine were eluted, treated with β-glucuronidase, and again chromatographed. No TNG was detected. However, 10.0% of the administered radioactivity was 1,2-DNG, 3.5% was 1,3-DNG, 1.5% was MNGs, 6.9% was glycerol, and 6.2% was unidentified polar material. This material had an R_f of 0.0 or 0.6 with the n-butanol:acetic acid:water solvent system. These results suggested the presence of glucuronide conjugates of DNGs and MNGs but not TNG.

8. 1,2-DNG

A 10% solution of 1,2-DNG in peanut oil was used in the acute toxicity studies. The acute oral LD_{50} ± S.E. (95% confidence limits) in male and female rats were 1,533 ± 150 (1,063-1,852) and 1,423 ± 176 (935-1,767) mg/kg, respectively; in male and female mice were 1,221 ± 131 (787-1,631) and 1,440 ± 130 (1,217-2,328) mg/kg, respectively. All treated animals exhibited ataxia and a reduced respiratory rate within 1 hour of dosing. The animals that survived the dose usually began to recover within

6 to 8 hours after dosing and recovery was usually complete within 24 hours after dosing. No gross pathology attributable to the treatment was noted in the animals that died. Primary skin and eye irritation tests in rabbits indicated that 1,2-DNG was not irritating to the skin or the eye. Dermal sensitivity tests in guinea pigs were not performed.

The distribution and excretion of radioactivity in 1,2-DNG-1,3-^{14}C treated rats are summarized in Table 17. About 85% of an oral dose was absorbed in 24 hours. The radioactivity recovered in the gastrointestinal tract and the feces averaged 56.4% and 14.2% at the end of 4 and 24 hours, respectively. Most of the absorbed radioactivity was excreted in urine and expired air, averaging 21.1% and 5.7% at 4 hours and 50.5% and 20.0% at 24 hours, respectively. The liver contained 3.8% of the administered radioactivity at 4 hours and that amount of radioactivity remained at 24 hours. Skeletal muscle, blood, kidneys, brain, lungs, and spleen contained 0.1% to 7.7% of the radioactivity at 4 hours and less than 0.1% to 1.7% at 24 hours. The tissue to plasma radioactivity ratios indicated some retention of radioactivity in the liver, kidneys, and lungs.

TLC analysis of radioactivity in the 24-hour urine from 1,2-DNG-1,3-^{14}C treated rats is summarized in Table 18. The parent compound represented only 1.0% of the administered dose. The free MNGs and polar components represented 15.4% and 34.1% of the administered radioactivity, respectively. The polar components were eluted and treated with β-glucuronidase. TLC analysis of the hydrolysate revealed that 15.3% of the administered radioactivity was the parent compound 1,3-DNG, 3.2% was MNGs, 8.7% was glycerol, and 6.9% was not identified. The unidentified polar material had an R_f of 0.0 or 0.6 with the n-butanol:acetic acid: water solvent system. This result suggested the presence of 1,2-DNG-glucuronide and MNG-glucuronides in the urine.

9. 1,3-DNG

A 10% solution of 1,3-DNG in peanut oil was used in the acute toxicity studies. The acute oral LD_{50} ± S.E. (95% confidence limits) in male and female rats were 1,751 ± 192 (1,335-2,248) and 1,065 ± 118 (813-1,367) mg/kg, respectively; in male and female mice were 676 ± 68 (546-851) and 688 ± 54 (582-836) mg/kg, respectively. The toxic signs included ataxia and a depressed respiration rate. Death occurred within 72 hours in rats and within 5 days in mice. No gross pathology attributable to the treatment was observed in the animals that died. Primary skin and eye irritation tests in rabbits were negative after treatment with 1,3-DNG. Dermal sensitivity tests in guinea pigs were not performed.

The distribution and excretion of radioactivity in 1,3-DNG-1,3-^{14}C treated rats are summarized in Table 19. About 75 to 85% of an oral dose was absorbed in 24 hours. The radioactivity recovered in the

gastrointestinal tract and the feces averaged 66.5% at 4 hours and 15.9% at 24 hours. The majority of the absorbed radioactivity was excreted in urine and expired air, averaging 6.9% and 7.5% at 4 hours and 27.7% and 25.1% at 24 hours, respectively. The liver contained 9.3% of the radioactivity at 4 hours and 7.1% at 24 hours. Small but significant amounts were also found in other tissues. The tissue to plasma radioactivity ratios indicated high retention in the liver and kidneys and some retention in the lungs, spleen, and skeletal muscle.

TLC analysis of radioactivity in the 24-hour urine from 1,3-DNG-1,3-^{14}C treated rats is summarized in Table 20. Only a small amount of the 1,3-DNG-1,3-^{14}C, averaging 0.2% of the dose, was excreted unchanged in the urine. Free 1-MNG and polar components represented 5.0% and 22.5% of the administered dose, respectively. The polar components were eluted and treated with β-glucuronidase. TLC analysis of the resulting hydrolysate revealed that 4.0% of the administered radioactivity was the unchanged 1,3-DNG, 1.1% was 1-MNG, 9.2% was glycerol, and 8.2% was not identified. The unidentified polar material had R_f of 0.0 or 0.6 with the n-butanol:acetic acid:water solvent system. As for the 1,2-DNG, this result suggested the presence of the 1,3-DNG-glucuronide and MNG-glucuronide in the urine.

10. 1-MNG

A 10% emulsion of 1-MNG in peanut oil and water was used in the acute toxicity studies. The acute oral LD_{50} ± S.E. (95% confidence limits) in male and female rats were 701 ± 31 (630-769) and 339 ± 39 (240-413) mg/kg, respectively; in male and female mice were 2,408 ± 683 (1,481-5,698) and 1,433 ± 310 (940-2,463) mg/kg, respectively. The toxic signs were ataxia and aphagia. Death occurred in 3 to 6 days, depending upon the dose given. No apparent gross pathology was noted in any of the animals that died. The primary skin and eye irritation tests in rabbits indicated that 1-MNG was not an irritant. Dermal sensitivity test in guinea pigs was not performed.

The distribution and excretion of radioactivity in 1-MNG-1,3-^{14}C treated rats are summarized in Table 21. About 70 to 80% of an oral dose was absorbed in 24 hours. The radioactivity recovered in the gastrointestinal tract and the feces averaged 33.8% at 4 hours and 17.8% at 24 hours. The majority of the absorbed radioactivity was excreted in the urine and expired air, averaging 18.9% and 9.2% at 4 hours and 29.0% and 30.5% at 24 hours, respectively. A significant amount of radioactivity averaging 4.2% of the dose was found in the liver at 4 hours and the amount was 2.9% at 24 hours. Small but significant amounts of radioactivity were found in the blood and various tissues. The tissue to plasma radioactivity ratios indicated some retention of radioactivity in the liver, kidneys, spleen, and lungs.

TLC analysis of radioactivity in the 24-hour urine from 1-MNG-1,3-^{14}C treated rats is summarized in Table 22. A significant amount of the parent compound averaging 13.3% of the dose was detected unchanged in the urine. Urinary glycerol and unidentified polar components represented 10.1% and 5.6% of the administered dose, respectively. The unidentified components did not migrate in the n-butanol:acetic acid:water solvent system.

11. 2-MNG

A 10% emulsion of 2-MNG in peanut oil and water was used in the acute toxicity studies. The acute oral LD_{50}s of 2-MNG in male and female rats and in male and female mice were over 5,000 mg/kg. Primary skin and eye irritation tests in rabbits indicated that it was not an irritant. Dermal sensitivity test in guinea pigs was not performed.

The distribution and excretion of radioactivity in 2-MNG-1,3-^{14}C treated rats are summarized in Table 23. About 60 to 70% of an oral dose was absorbed in 24 hours. The radioactivity recovered in the gastrointestinal tract and the feces averaged 54.0% at 4 hours and 33.3% at 24 hours. The majority of the absorbed radioactivity was excreted in the urine, averaging 28.3% at 4 hours and 48.8% at 24 hours. There was only 1.6% of radioactivity in the expired air at 4 hours and 7.2% at 24 hours. A small but significant amount of radioactivity was found in the liver. Small amounts of radioactivity were also found in the blood and other tissues at 4 hours and the amounts decreased greatly at 24 hours. The tissue to plasma radioactivity ratios suggested the retention of small amounts of radioactivity in all tissues.

TLC analysis of radioactivity in the 24-hour urine from 2-MNG-1,3-^{14}C treated rats is summarized in Table 24. Unchanged 2-MNG represented 24.4% of the administered dose. Urinary glycerol and unidentified polar components represented 21.7% and 2.7%, respectively. The unidentified components did not migrate in the n-butanol:acetic acid:water solvent system.

12. Nitrocellulose

Nitrocellulose was prepared as a 5% suspension in water. Each dose was divided in half and given 30 minutes apart due to the large volume necessary to administer a dose of 5,000 mg/kg. The LD_{50}s for nitrocellulose in male and female rats and in male and female mice were greater than 5,000 mg/kg. No toxic signs were noted in animals receiving the highest dose of nitrocellulose. Two of 10 male mice given 5,000 mg/kg died without any apparent gross lesions. No other animals died from any doses.

Primary skin and eye irritation tests in rabbits were negative with nitrocellulose. Dermal sensitivity tests in guinea pigs were not performed.

A sufficient amount of ^{14}C-labeled nitrocellulose has not been obtained for the absorption and disposition study. First, ^{14}C-labeled cellulose is obtained by growing cotton plants in synthetic medium containing ^{14}C-glucose. The harvested labeled cellulose from the plants is subsequently nitrated to obtain the ^{14}C-labeled nitrocellulose. The first batch of 14.25 µCi of ^{14}C-cellulose was obtained. Additional amounts of ^{14}C-cellulose will be obtained during August-September 1975.

13. White Phosphorus

A 0.1% solution of white phosphorus in peanut oil was used for these studies. White phosphorus was by far the most toxic of all the compounds tested. The acute oral LD_{50}s ± S.E. (95% confidence limits) in male and female rats were 3.76 ± 0.22 (3.35-4.43) and 3.03 ± 0.15 (2.70-3.38) mg/kg, respectively; in male and female mice, 4.85 ± 0.21 (4.34-5.35) and 4.82 ± 0.38 (3.87-5.63) mg/kg, respectively. Death occurred in the treated animals over a period of several days. Animals that died were found to have large yellow nutmeg livers.

Primary skin irritation and eye irritation tests were negative with a 0.1% solution of white phosphorus. Higher concentrations were not tested because of the possibility of producing systemic toxicity. Dermal sensitivity test in guinea pigs was not performed.

The distribution and excretion of radioactivity in ^{32}P-labeled white phosphorus treated rats are summarized in Table 25. About 60 to 65% of an oral dose was absorbed and the absorption was essentially completed in 24 hours. The amount of radioactivity remaining in the gastrointestinal tract was 57.0%, 15.3%, and 1.7% of the administered dose at the end of 4 hours, 1 day, and 5 days, respectively. During these same time periods, 2.0%, 16.6%, and 33.0% of the radioactivity was recovered in the feces. The majority of the absorbed radioactivity was excreted in the urine, averaging 17.1% of the administered dose at 4 hours, 34.5% at 1 day, and 46.7% at 5 days. The liver contained the highest amount of radioactivity, representing 16.1% of the dose at 4 hours, 16.9% at 1 day, and 6.3% at 5 days. Significant amounts of radioactivity were also found in the blood and skeletal muscle. The radioactivity in the blood represented 6.1%, 4.1%, and 1.7% of the administered dose at the end of 4 hours, 1 day, and 5 days, respectively. The amount in the muscle, averaging 4.0%, 5.5%, and 6.0% at 4 hours, 1 day, and 5 days, respectively, was probably due to the large mass of muscle in the body.

The ratios of radioactivity in the various tissues, relative to plasma, are summarized in Table 26. At 4 hours, the radioactivity ratios were in the order of liver > kidneys > lungs > spleen > bone > muscle > brain. The radioactivity concentration in the blood decreased slowly and consistently; whereas the radioactivity in various tissues remained high or decreased only slightly. This resulted in large increases in the tissue to plasma radioactivity ratios in all tissues. Another group of rats was treated with ^{32}P-labeled white phosphorus for 5 consecutive days to compare the amount of radioactivity in each tissue 24 hours after the last dose with that in the tissue 24 hours after a single dose. The results of these experiments are given in Table 27. All the tissues from rats receiving 5 daily doses contained 4.1 to 10.5 times as much radioactivity as those receiving a single dose. This result indicated an accumulation of radioactivity in all tissues.

TLC analysis of radioactivity in the urine from rats treated with ^{32}P-labeled white phosphorus is shown in Figure 8. There were two major peaks of radioactivity with the butanol:methanol:water system. Both components migrated in this solvent system. The first component had an R_f value of 0.25 which is in the region where inorganic phosphate migrates in this solvent system. The other radioactive component was less polar, having an R_f value of 0.45.

TLC analysis of radioactivity in the liver extracts also demonstrated two radioactive components, as shown in Figure 9. These components had R_f values similar to those appearing in the urine. The first component had an R_f value of 0.19; the other component had an R_f value of 0.48. Again, the first component is in the region where inorganic phosphate migrates in the solvent system.

IV. DISCUSSION AND CONCLUSIONS

A. Acute Oral Toxicity in Rats

1. TNTs

In male rats, the acute oral LD_{50} of TNT was 1,010 mg/kg. Among the DNT isomers, 2,4-DNT, 2,5-DNT, and 2,6-DNT were more toxic. The LD_{50}s ranged from 535 to 616 mg/kg. The LD_{50} of 2,3-DNT and 3,4-DNT approached that of the TNT. The slope for the LD_{50} of 2,3-DNT was rather large. In female rats, the LD_{50} of TNT was 820 mg/kg, considerably smaller than that in the males. On the other hand, the LD_{50} of 2,6-DNT in female rats was 795 mg/kg, considerably larger than that in the males. The LD_{50}s of the other DNT isomers in female rats ranged from 517 to 911 mg/kg.

2. TNGs

In male rats, the acute oral LD_{50} of TNG was 822 mg/kg. Both 1,2-DNG and 1,3-DNG were less toxic than TNG. The LD_{50}s ranged from 1,533 to 1,751 mg/kg. 1-MNG was more toxic than TNG with an LD_{50} of 701 mg/kg; but 2-MNG was relatively nontoxic with an LD_{50} larger than 5,000 mg/kg. In female rats, the LD_{50} of TNG was 884 mg/kg, similar to that in the males. The LD_{50} of both the 1,2-DNG and the 1,3-DNG was about the same as or slightly larger than that of the TNG. 1-MNG was much more toxic in the female rats; its LD_{50} was 339 mg/kg. As seen in the male rats, 2-MNG was relatively nontoxic in the females with an LD_{50} of larger than 5,000 mg/kg.

3. Nitrocellulose and White Phosphorus

The nitrocellulose was relatively nontoxic in rats. The LD_{50} was more than 5,000 mg/kg. White phosphorus was by far the most toxic compound studied with an LD_{50} of 3.76 and 3.03 mg/kg in male and female rats, respectively.

B. Acute Oral Toxicity in Mice

1. TNTs

The acute toxicity of TNT in mice was similar to that in rats. The LD_{50}s of TNT in male and female mice were 1,014 and 1,009 mg/kg, respectively. As seen in the rats, both 2,5-DNT and 2,6-DNT were more toxic than TNT in mice. The toxicity of 3,4-DNT approached that of TNT and the toxicity of 2,3-DNT was slightly less than that of TNT in mice. In contrast to rats, the toxicity of 2,4-DNT in mice was considerably less than that of TNT; its LD_{50} was 1,954 mg/kg. In addition, the LD_{50}s of both 2,3-DNT and 2,4-DNT in male mice were larger than those in female mice.

2. TNGs

The acute toxicity of TNG in mice was slightly less than that in rats. The LD_{50}s of TNG in male and female mice were 1,188 and 1,055 mg/kg, respectively. The toxicity of 1,2-DNG in mice was about the same as that of TNG. In contrast to rats, 1,3-DNG was more toxic and 1-MNG was less toxic than TNG in mice. The LD_{50}s of 1,3-DNG in male and female mice was 676 and 688 mg/kg, respectively; and those of 1-MNG in male and female mice were 2,408 and 1,433 mg/kg, respectively. The confidence limits for the LD_{50} of 1-MNG in mice were very wide, so the LD_{50} differences between males and females were not considered significant. As seen in the rats, 2-MNG was relatively nontoxic in mice with an LD_{50} of larger than 5,000 mg/kg.

3. Nitrocellulose and White Phosphorus

In mice, nitrocellulose was also relatively nontoxic with an LD_{50} of more than 5,000 mg/kg. The white phosphorus was also by far the most toxic compound studied in mice with an LD_{50} of 4.85 and 4.82 mg/kg in the males and the females, respectively.

C. Toxic Effects

Rats and mice receiving toxic oral doses of TNT and 3,4-DNT exhibited coordinated and symmetrical convulsions which began 5 to 30 minutes after dosing. Animals receiving lethal doses of these compounds died of respiratory paralysis associated with the convulsive episodes. If the animals were able to regain their respiratory function after the convulsions, they survived the dose. Generally, the convulsions subsided in the survivors 1 to 2 hours after dosing. Animals that died after 3,4-DNT treatment developed rigor mortis immediately. The animals that received toxic doses of TNT, 3,4-DNT, and the other DNTs exhibited ataxia, respiratory depression, and often a transient cyanosis which lasted 2 to 4 hours. Death usually occurred within 24 hours. Gross lesions attributable to the treatment were not noted in any animal.

A red colored urine appeared in both rats and mice within 5 to 15 minutes after dosing with TNT and a bright yellow colored urine appeared in both species after treatment with 3,4-DNT within the same time. Excretion of these colored urines continued for several hours after treatment. Fur and bedding were heavily stained for several days after treatment. Occasionally, a yellow colored urine also occurred in rats and mice after treatment with 2,5-DNT. It did not appear immediately after treatment and there appeared to be less staining of the fur and bedding. These colored urines indicated the presence of some metabolites.

Rats and mice treated with toxic doses of TNG, DNGs, and 1-MNG became cyanotic, exhibited ataxia, and had respiratory depression within the first hour after dosing. Death of animals treated with TNG and 1,2-DNG occurred during the first day. The cause of death was respiratory depression. The animals that survived usually recovered by the end of the first day. However, animals treated with 1,3-DNG or 1-MNG died in 3 to 6 days, depending upon the dose and species. These animals stopped eating in addition to the toxic signs described above. No apparent gross lesions attributable to the treatment were noted in animals that died.

Rats and mice treated with toxic doses of white phosphorus had depression and anorexia. Death in both species occurred over a period of several days. The livers of animals that died were enlarged and yellow.

D. Primary Skin and Eye Irritation

The primary skin irritation test in rabbits indicated that 2,5-DNT was a moderate irritant. Both the intact and abraided skin treated with the compound underwent necrosis in 24 hours without edema. TNT, the other DNTs (2,3-DNT, 2,4-DNT, 2,6-DNT, and 3,4-DNT), and TNG were found to be mild irritants. Both DNGs, both MNGs, nitrocellulose, and white phosphorus were not irritating to the rabbit skin in the concentrations tested. The primary eye irritation test indicated that none of the test compounds was an eye irritant.

A red stain was apparent on the skin under the patches or around the eye in rabbits treated with TNT; a yellow stain was apparent under the patches or around the eye in rabbits treated with 3,4-DNT. These color stains were similar to those seen in the urine of both rats and mice after oral administration of these compounds.

E. Dermal Sensitivity

Topical application of TNT as a 4.12% solution in peanut oil and TNG as a 7.5% solution in 25% peanut oil containing 67.5% lactose produced 40% response according to the guinea pig "maximization test" procedure.[6/] They were considered to be moderate sensitizing agents. 2,6-DNT as a 5% solution in peanut oil producing 20% response was considered to be a mild sensitizing agent. Dermal sensitization tests on other DNTs (2,3-DNT, 2,4-DNT, 2,5-DNT, and 3,4-DNT) were negative. Cross sensitization was not considered in these studies.

F. Disposition and Metabolism

1. Nitrotoluenes

a. Absorption and distribution: The nitrotoluenes (ring-UL-^{14}C) were readily absorbed after oral administration in the rat. The absorption was essentially completed in 24 hours with approximately 60 to 90% of the administered doses. The extents of absorption were in the following order: 2,4-DNT, 3,4-DNT > TNT, 2,5-DNT > 2,3-DNT, 2,6-DNT. These values may not, however, reflect the true absorption, since biliary excretion may play an important role in the metabolism of these nitrotoluenes. Biliary excretion experiments are currently being performed and will be reported upon completion.

The liver and kidneys contained small but significant amounts of radioactivity. Very small amounts of radioactivity were also found in the other tissues, including brain, lungs, skeletal muscle, and/or spleen. The tissue to plasma radioactivity ratios indicated that the nitrotoluenes and/or their metabolites were, in general, readily taken into most tissues, as indicated by a ratio greater than unity. A comparison of the radioactivity ratios at 4 and 24 hours indicated that the radioactivity was retained in most tissues, especially in the liver and kidneys. The retention of radioactivity in tissues was more apparent with 2,4-DNT, 2,5-DNT, 2,6-DNT, and 3,4-DNT. In addition, the retention of radioactivity occurred in the brain treated with 2,4-DNT. This retention of radioactivity in various tissues correlated with greater toxicity of these four DNTs in rats.

b. Excretion: Most of the absorbed radioactivity from oral administration of nitrotoluenes was excreted in the urine. It indicated that these compounds were extensively metabolized in the body since nitrotoluenes are water insoluble. The aromatic ring of these nitrotoluenes remained intact. Only a negligible amount of radioactivity was recovered in the expired air.

c. Metabolism: TLC analysis of the urine from treated rats indicated that the nitrotoluenes were metabolized extensively. Unchanged parent compounds were not present in the urine. The urinary metabolites consisted of one or two classes of polar metabolites. Brain extracts from 3,4-DNT treated rats contained a radioactive metabolite which had an R_f value similar to the parent compound. The presence of this metabolite in the brain coincided with 3,4-DNT induced convulsions. TLC analysis of brain extracts from TNT treated rats also revealed a radioactive nonpolar metabolite. However, this did not correspond to TNT.

2. Nitroglycerins

a. Absorption and distribution: The nitroglycerins (NG-1,3-^{14}C) were readily absorbed after oral administration in the rat. The absorption was essentially completed in 24 hours with approximately 64 to 90% of the administered dose. The extent of absorption was about the same for TNG, 1,2-DNG, 1,3-DNG, and 1-MNG, with slightly less for 2-MNG. The slightly less absorption for 2-MNG correlates with the lower oral toxicity of this compound. How much biliary excretion contributes to the metabolism of nitroglycerins is not known. Biliary excretion experiments are currently being performed.

The liver contained relatively large amounts of radioactivity, averaging 2 to 9% of the administered dose. The amount of radioactivity in the liver was essentially the same at 4 and 24 hours. Only small amounts of radioactivity were found in the other tissues. The tissue to plasma

radioactivity ratios for liver, kidneys, spleen, brain, lungs, and/or skeletal muscle were greater than unity. A comparison of the radioactivity ratios at 4 and 24 hours indicated that the radioactivity was retained in most tissues, especially the liver and kidneys.

b. Excretion: Most of the absorbed radioactivity from oral administration of the nitroglycerins (NG-1,3-^{14}C) was excreted in the urine and in the expired air. The excretion of radioactivity in the urine for the various nitroglycerins averaged 30 to 50% of the dose. TNG and the DNGs are not water soluble. The excretion of radioactivity in the urine indicated their metabolism to more polar components. The radioactivity recovered in the expired air averaged 20 to 30% for TNG, the DNGs and 1-MNG. For 2-MNG, only 7% of the administered radioactivity was excreted in the expired air.

c. Metabolism: The large amounts of radioactivity in the expired air indicated that the glycerol portion of the nitroglycerins was extensively metabolized. After administration of TNG or DNGs, only small amounts of the unchanged parent compounds were found in the urine. The urinary metabolites consisted of free MNGs, glycerol, and other polar metabolites, including glucuronides. This indicated that TNG and the DNGs were metabolized by denitration and conjugation to produce water soluble metabolites. The MNGs are relatively water soluble. Considerable amounts of the unchanged MNGs were excreted in the urine. In addition, the MNGs were metabolized to glycerol and some unidentified polar components.

3. White Phosphorus

a. Absorption and distribution: White phosphorus was moderately absorbed after oral administration in the rat. The absorption was essentially completed in 24 hours with approximately 60 to 65% of the administered dose. The actual amount absorbed, however, may be higher since the role of biliary excretion in the metabolism of white phosphorus is not known.

The liver contained large amounts of radioactivity, averaging 16% at 4 hours, 16% at 1 day, and 6% at 5 days. This large amount of radioactivity is consistent with white phosphorus poisoning since enlarged nutmeg liver was observed in the acute oral toxicity study. The skeletal muscle also contained a large percentage of the dose. This, however, is probably due to the large mass of muscle in the body. Only small amounts of ^{32}P were found in the other tissues. The tissue to plasma radioactivity ratios after a single dose and the ratio of radioactivity in the tissues after repeated administration relative to a single dose indicated an accumulation of radioactivity in all tissues examined.

b. Excretion: Most of the absorbed radioactivity from oral administration of white phosphorus was excreted in the urine. This suggested that the white phosphorus was metabolized in the body since white phosphorus is, itself, only sparingly soluble in water.

c. Metabolism: TLC analysis of urine indicated the urinary metabolites consisted of two classes of compounds, one of which corresponded to inorganic phosphate. The other class of compound was much more nonpolar and was suggestive of an organic phosphate(s). TLC analysis of liver extracts also demonstrated two classes of metabolites with properties similar to those in the urine.

V. SUMMARY

Relatively pure TNT and its dinitroisomers (2,3-DNT, 2,4-DNT, 2,5-DNT, 2,6-DNT, and 3,4-DNT), TNG and its dinitroisomers (1,2-DNG and 1,3-DNG) and mononitroisomers (1-MNG and 2-MNG), nitrocellulose, and white phosphorus were obtained commercially or synthesized for acute oral toxicity, primary skin and eye irritation, and dermal sensitization studies. ^{14}C-labeled nitrotoluenes and nitroglycerins and ^{32}P were used for study of disposition and metabolism.

The acute oral LD_{50}s of TNT and the DNTs in male rats were determined to be between 535 mg/kg and 1,102 mg/kg, and their toxicity was in the decreasing order of 2,6-DNT, 2,4-DNT, 2,5-DNT, 3,4-DNT, TNT, and 2,3-DNT. In female rats, the LD_{50}s were between 517 mg/kg and 911 mg/kg, and their toxicity was in the decreasing order of 2,5-DNT, 2,4-DNT, 2,6-DNT, 3,4-DNT, TNT, and 2,3-DNT. In male mice, the LD_{50}s were between 621 mg/kg and 1,954 mg/kg, and their toxicity was in the decreasing order of 2,6-DNT, 2,5-DNT, 3,4-DNT, TNT, 2,3-DNT, and 2,4-DNT. In female mice, the LD_{50}s were between 659 mg/kg and 1,340 mg/kg, and their toxicity was in the decreasing order of 2,5-DNT, 3,4-DNT, 2,6-DNT, TNT, 2,3-DNT, and 2,4-DNT. Rats and mice receiving toxic doses exhibited ataxia, respiratory depression, and often a transient cyanosis. Death occurred within 24 hours. In addition, TNT and 3,4-DNT caused coordinated, symmetrical convulsions within 5 to 30 minutes, and death due to respiratory paralysis associated with convulsions. A red colored urine appeared in TNT treated animals within 15 minutes, and a bright yellow colored urine in 3,4-DNT treated animals. Occasionally, a yellow colored urine appeared in 2,5-DNT treated animals.

The acute oral LD_{50}s of TNG, the DNGs, and 1-MNG in male rats were determined to be between 701 mg/kg and 1,751 mg/kg, and their toxicity was in the decreasing order of 1-MNG, TNG, 1,2-DNG, and 1,3-DNG. In female rats, the LD_{50}s were between 339 mg/kg and 1,423 mg/kg, and their toxicity was in the decreasing order of 1-MNG, TNG, 1,3-DNG, and 1,2-DNG.

In male mice, the LD_{50}s were between 676 mg/kg and 2,408 mg/kg, and their toxicity was in the decreasing order of 1,3-DNG, TNG, 1,2-DNG, and 1-MNG. In female mice, the LD_{50}s were between 688 mg/kg and 1,440 mg/kg, and their toxicity was in the decreasing order of 1,3-DNG, TNG, 1-MNG, and 1,2-DNG. 2-MNG was relatively nontoxic to both rats and mice with an LD_{50} of > 5,000 mg/kg. Rats and mice treated with toxic doses of TNG, DNGs, and 1-MNG became cyanotic, exhibited ataxia, and had respiratory depression within the first hour. Death, due to respiratory depression, occurred during the first day in TNG and 1,2-DNG treated animals and between 3 and 6 days in 1,3-DNG and 1-MNG treated animals.

Nitrocellulose was relatively nontoxic to both rats and mice. The LD_{50} in both species was > 5,000 mg/kg.

White phosphorus was by far the most toxic compound tested. The LD_{50}s in male and female rats were 3.76 mg/kg and 3.03 mg/kg, respectively, and in male and female mice were 4.85 mg/kg and 4.82 mg/kg, respectively. Toxic doses of white phosphorus caused depression and anorexia in both rats and mice. Deaths occurred over a period of several days. Animals that died had enlarged yellow nutmeg livers.

The primary skin irritation test in rabbits indicated that 2,5-DNT was a moderate irritant. TNT, the other DNTs (2,3-DNT, 2,4-DNT, 2,6-DNT, and 3,4-DNT), and TNG were mild irritants. Both DNGs, both MNGs, nitrocellulose, and white phosphorus were not irritating to the rabbit skin. None of these compounds was an eye irritant. Like the urine, a red stain was present on the skin under the patches or around the eye in rabbits treated with TNT; and a yellow stain, in rabbits treated with 3,4-DNT.

Topical applications of TNT as a 4.12% solution in peanut oil and of TNG as a 4.85% solution in 50% peanut oil containing 45% lactose to guinea pigs were found to be moderate sensitizing agents; and 2,6-DNT as a 5% solution in peanut oil was found to be a mild sensitizing agent. Dermal sensitization tests on 2,3-DNT, 2,4-DNT, 2,5-DNT, and 3,4-DNT were negative.

The nitrotoluenes (ring-UL-^{14}C) were well absorbed after oral administration in the rat. The absorption was essentially completed in 24 hours with approximately 60 to 90% of the administered dose. The extent of absorption was in the following order: 2,4-DNT, 3,4-DNT > TNT, 2,5-DNT > 2,3-DNT, 2,6-DNT. The liver and kidneys contained small but significant amounts of radioactivity. Very small amounts of radioactivity were also found in other tissues. The tissue to plasma radioactivity ratios indicated that the radioactivity was retained in most tissues, especially in the liver and kidneys. Most of the absorbed radioactivity was excreted in the urine. Practically no radioactivity was recovered in the expired air. TLC analysis of the urine from treated rats indicated

that unchanged parent compounds were not present in the urine. The urinary metabolites consisted of one or two classes of polar components. Brain extracts from 3,4-DNT treated rats contained a radioactive metabolite which had an R_f value similar to the parent compound. The presence of this metabolite in the brain coincided with 3,4-DNT induced convulsions. TLC analysis of brain extracts from TNT treated rats also revealed a radioactive nonpolar metabolite. However, this metabolite did not correspond to TNT.

The nitroglycerins (NG-1,3-^{14}C) were also well absorbed after oral administration in the rat. The absorption was essentially completed in 24 hours with approximately 64 to 90% of the administered dose. The extent of absorption was about the same for TNG, 1,2-DNG, 1,3-DNG, and 1-MNG with slightly less for 2-MNG. The liver contained relatively large amounts of radioactivity, averaging 2 to 9% of the administered dose. The amount of radioactivity in the liver was essentially the same at 4 and 24 hours. Only small amounts of radioactivity were found in the other tissues. A comparison of the radioactivity ratios at 4 and 24 hours indicated that the radioactivity was retained in most tissues, especially the liver and kidneys. Most of the absorbed radioactivity was excreted in the urine and in the expired air. The excretion of radioactivity in the urine averaged 30 to 50% of the dose. The radioactivity recovered in the expired air averaged 20 to 30% for TNG, the DNGs, and 1-MNG, and 7% for 2-MNG. The large amount of radioactivity in the expired air indicated that the glycerol portion of the nitroglycerins was extensively metabolized. After administration of TNG or the DNGs, the urinary metabolites consisted of free MNGs, glycerol, and other polar metabolites, including glucuronides. This indicated that TNG and the DNGs were metabolized by denitration and conjugation to produce water-soluble metabolites. After administration of the MNGs, considerable amounts of the unchanged parent compound were excreted in the urine. In addition, the MNGs were metabolized to glycerol and some unidentified polar components.

The white phosphorus was moderately absorbed after oral administration in the rat. The absorption was essentially completed in 24 hours with approximately 60 to 65% of the administered dose. The liver contained large amounts of radioactivity averaging 16% at 4 hours, 16% at 1 day, and 6% at 5 days. The skeletal muscle also contained a large percentage of the dose, probably due to the large mass of muscle in the body. Only small amounts of ^{32}P were found in the other tissues. The tissue to plasma radioactivity ratios indicated an accumulation of radioactivity in all tissues examined. Most of the absorbed radioactivity was excreted in the urine. TLC analysis of urine indicated that the urinary metabolites consisted of two classes of compounds, one of which corresponded to inorganic phosphate. The other class was more nonpolar and suggested an organic phosphate. TLC analysis of liver extracts also demonstrated two classes of metabolites with properties similar to those found in the urine.

TABLE 1

SPECIFIC ACTIVITIES OF TOLUENES-(RING-UL-^{14}C), NITROGLYCERINS-1,3-^{14}C, AND ^{32}P-WHITE PHOSPHORUS USED FOR THE METABOLISM STUDIES

Compound	Specific Activity
TNT	4.49 mCi/mM
2,3-DNT	3.02 mCi/mM
2,4-DNT	3.55 mCi/mM
2,5-DNT	3,19 mCi/mM
2,6-DNT	4.90 mCi/mM
3,4-DNT	3.55 mCi/mM
TNG	53.25 mCi/mM
1,2-DNG	36.67 mCi/mM
1,3-DNG	36.67 mCi/mM
1-MNG	36.67 mCi/mM
2-MNG	36.67 mCi/mM
P	0.53 mCi/mg

TABLE 2

ACUTE ORAL TOXICITIES (mg/kg) OF VARIOUS MUNITION COMPOUNDS IN MALE AND FEMALE RATS

Compounds	Males			Females		
	LD_{50} ± S.E.	95% Confidence Limits	Slope ± S.E.	LD_{50} ± S.E.	95% Confidence Limits	Slope ± S.E.
TNT	1,010 ± 41	922 - 1,108	5.96 ± 1.50	820 ± 32	747 - 889	5.68 ± 1.41
2,3-DNT	1,102 ± 20	1,011 - 1,169	15.46 ± 6.84	911 ± 65	584 - 1,049	3.25 ± 1.37
2,4-DNT	568 ± 59	434 - 705	2.25 ± 0.61	650 ± 49	520 - 743	3.53 ± 0.94
2,5-DNT	616 ± 34	532 - 707	4.20 ± 1.37	517 ± 25	477 - 575	5.56 ± 1.85
2,6-DNT	535 ± 58	397 - 646	1.79 ± 0.41	795 ± 22	744 - 844	8.48 ± 2.14
3,4-DNT	907 ± 42	815 - 1,011	4.78 ± 1.28	807 ± 33	721 - 874	4.67 ± 1.23
TNG	822 ± 54	700 - 953	3.35 ± 0.95	884 ± 61	763 - 1,055	3.33 ± 0.94
1,2-DNG	1,533 ± 150	1,063 - 1,852	2.37 ± 0.83	1,423 ± 1.76	935 - 1,767	2.23 ± 0.68
1,3-DNG	1,751 ± 192	1,335 - 2,248	1.98 ± 0.57	1,065 ± 118	813 - 1,367	2.52 ± 0.71
1-MNG	701 ± 31	630 - 769	4.28 ± 1.05	339 ± 39	240 - 413	2.75 ± 0.74
2-MNG	> 5,000	--	--	> 5,000	--	--
Nitrocellulose	> 5,000	--	--	> 5,000	--	--
White Phosphorus	3.76 ± 0.22	3.35 - 4.43	4.44 ± 1.21	3.03 ± 0.15	2.70 - 3.38	5.07 ± 1.32

TABLE 3

ACUTE ORAL TOXICITIES (mg/kg) OF VARIOUS MUNITION COMPOUNDS IN MALE AND FEMALE MICE

Compounds	Males			Females		
	LD_{50} ± S.E.	95% Confidence Limits	Slope ± S.E.	LD_{50} ± S.E.	95% Confidence Limits	Slope ± S.E.
TNT	1,014 ± 52	905 - 1,163	3,47 ± 1.06	1,009 ± 54	880 - 1,117	3.86 ± 0.88
2,3-DNT	1,372 ± 34	1,285 - 1,441	8.53 ± 2.26	1,089 ± 32	1,029 - 1,175	7.96 ± 2.03
2,4-DNT	1,954 ± 68	1,848 - 2,178	4.50 ± 1.15	1,340 ± 67	1,205 - 1,500	4.15 ± 0.93
2,5-DNT	652 ± 28	585 - 712	5.05 ± 1.29	659 ± 12	633 - 690	12.97 ± 3.51
2,6-DNT	621 ± 51	488 - 721	3.25 ± 0.87	807 ± 35	735 - 893	5.93 ± 1.51
3,4-DNT	859 ± 37	787 - 958	4.19 ± 1.10	747 ± 26	702 - 821	5.74 ± 1.28
TNG	1,188 ± 76	1,008 - 1,352	2.91 ± 0.75	1,055 ± 63	895 - 1,178	3.85 ± 0.98
1,2-DNG	1,221 ± 131	787 - 1,631	1.60 ± 0.66	1,440 ± 130	1,217 - 2,328	2.13 ± 0.85
1,3-DNG	676 ± 68	546 - 851	1.91 ± 0.46	688 ± 54	582 - 836	2.84 ± 0.76
1-MNG	2,408 ± 683	1,481 - 5,698	0.55 ± 0.15	1,433 ± 310	940 - 2,463	0.58 ± 0.14
2-MNG	> 5,000	--	--	> 5,000	--	--
Nitrocellulose	> 5,000	--	--	> 5,000	--	--
White Phosphorus	4.85 ± 0.21	4.34 - 5.35	6.35 ± 1.84	4.82 ± 0.38	3.87 - 5.63	2.91 ± 0.84

TABLE 4

PRIMARY SKIN IRRITATION OF VARIOUS MUNITION COMPOUNDS IN RABBITS

Compounds	Primary Irritation Score[a]
TNT	1.0[b]
2,3-DNT	1.78
2,4-DNT	0.25
2,5-DNT	3.80[c]
2,6-DNT	0.21
3,4-DNT	2.00[d]
TNG	0.46
1,2-DNG	< 0.2
1,3-DNG	< 0.2
1-MNG	< 0.2
2-MNG	< 0.2
Nitrocellulose	< 0.2
White Phosphorus	< 0.2
Peanut Oil (vehicle control)	0.33

a/ Six rabbits with intact and abraded skin in each test group. The compounds are classified as follows:
> 0.2 over controls is mild irritant
> 2.5 over controls is moderate irritant
> 5.0 over controls is severe irritant

b/ Red color under all patches at 24 hours.

c/ No edema was apparent but the entire area covered by the compound was undergoing necrosis in 24 hours in both the intact and abraded skin.

d/ Yellow color under all patches at 24 hours.

TABLE 5

PRIMARY EYE IRRITATION OF VARIOUS MUNITION COMPOUNDS IN RABBITS

Compounds	Result[a/]
TNT[b/]	Nonirritant
2,3-DNT	
2,4-DNT	
2,5-DNT	
2,6-DNT	
3,4-DNT[c/]	
TNG	
1,2-DNG	
1,3-DNG	
1-MNG	
2-MNG	
Nitrocellulose	
White Phosphorus	
Peanut Oil (vehicle control)	

a/ Six rabbits per test groups.
b/ Red color around the eye at 24 hours.
c/ Yellow color around the eye at 24 hours.

TABLE 6

DERMAL SENSITIVITY OF VARIOUS COMPOUNDS IN GUINEA PIGS

Compound	Number Responding	% Response	Sensitization
TNT	4/10	40%	moderate
2,3-DNT	0/10	0	none
2,4-DNT	0/10	0	none
2,5-DNT	0/10	0	none
2,6-DNT	2/10	20%	mild
3,4-DNT	0/10	0	none
TNG	4/10	40%	moderate

TABLE 7

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING A SINGLE DOSE OF TNT (RING-UL-^{14}C)

	% of Administered Dose		Tissue/Plasma Radioactivity Ratio[e]	
	30 Minutes[c]	24 Hours	30 Minutes	24 Hours
Gastrointestinal tract plus contents	-	20.7 ± 2.7	-	-
Feces	-	5.5 ± 1.1	-	-
Whole Blood[a]	0.2 ± 0.0[d]	0.6 ± 0.1	-	-
Expired Air	-	0.1 ± 0.0	-	-
Urine	0.2 ± 0.1	53.3 ± 3.1	-	-
Liver	0.3 ± 0.1	0.6 ± 0.0	3.5 ± 0.6	2.0 ± 0.1
Kidneys	0.1 ± 0.0	0.2 ± 0.0	2.4 ± 0.2	2.7 ± 0.2
Brain	0.1 ± 0.0	< 0.1	1.3 ± 0.2	0.3 ± 0.0
Lungs	-	< 0.1	-	0.9 ± 0.1
Skeletal Muscle[b]	-	1.0 ± 0.1	-	0.4 ± 0.0
Recovery		82.1 ± 3.0		

a/ Based on 7.0% of the body weight.
b/ Based on 40% of the body weight.
c/ The rats in the 30-minute group received the minimum lethal dose.
d/ Mean ± S.E. of three rats.
e/ Radioactivity in 1 ml or gm of wet tissue per radioactivity in 1 ml of plasma.

TABLE 8

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING A SINGLE DOSE OF 2,3-DNT (RING-UL-^{14}C)

	% of Administered Dose		Tissue/Plasma Radioactivity Ratio[d]	
	4 Hours	24 Hours	4 Hours	24 Hours[e]
Gastrointestinal tract plus contents	75.1 ± 11.1[c]	6.9 ± 1.7		
Feces	1.3 ± 0.7	32.1 ± 2.9		
Whole Blood[a]	0.3 ± 0.1	0.1 ± 0.0		
Expired Air	-	< 0.1		
Urine	20.7 ± 9.7	64.1 ± 2.1		
Liver	0.3 ± 0.1	0.3 ± 0.1	1.9 ± 0.1	2.9 ± 0.3
Kidneys	0.3 ± 0.1	< 0.1	8.7 ± 1.3	4.3 ± 0.0
Spleen	< 0.1	< 0.1	0.5 ± 0.1	0.7 ± 0.1
Brain	< 0.1	< 0.1	0.5 ± 0.1	0.3 ± 0.0
Lungs	< 0.1	< 0.1	0.7 ± 0.1	0.8 ± 0.1
Skeletal Muscle[b]	1.1 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
Recovery	99.3% ± 2.3	103.7% ± 0.9		

a/ Based on 7% of the body weight.
b/ Based on 40% of the body weight.
c/ Mean ± S.E. of three rats.
d/ Radioactivity in 1 ml or bm of wet tissue per radioactivity in 1 ml of plasma.
e/ Based on data from two rats.

TABLE 9

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING A SINGLE DOSE OF 2,4-DNT (RING-UL-^{14}C)

	% of Adminstered Dose		
	4 Hours	1 Day	5 Days
Gastrointestinal Tract Plus Contents	59.7 ± 3.3[c]	2.8 ± 1.5	0.1 ± 0.0
Feces	0.6 ± 0.3	9.1 ± 3.0	11.2[d]
Whole Blood[a]	0.2 ± 0.0	0.1 ± 0.0	< 0.1
Expired Air	-	0.1 ± 0.0	-
Urine	29.3 ± 3.3	75.9 ± 2.6	85.6[d]
Liver	0.6 ± 0.2	0.3 ± 0.1	0.1 ± 0.0
Kidneys	0.2 ± 0.0	< 0.1	< 0.1
Brain	< 0.1	< 0.1	< 0.1
Lungs	< 0.1	< 0.1	< 0.1
Skeletal Muscle[b]	0.6 ± 0.3	0.3 ± 0.1	0.2 ± 0.0
Recovery	91.2 ± 0.2	88.5 ± 2.6	97.2

a/ Based on 7.0% of the body weight.
b/ Based on 40% of the body weight.
c/ Mean ± S.E. of three rats.
d/ Pooled samples from three rats.

TABLE 10

TISSUE/PLASMA RATIOS OF RADIOACTIVITY IN RATS RECEIVING 2,4-DNT (RING-UL-^{14}C)

Tissue	Tissue/Plasma Radioactivity Ratio[a/]		
	4 Hours	1 Day	5 Days
Liver	3.6 ± 1.0[b/]	18.1 ± 0.7	30.3 ± 5.6
Kidneys	8.5 ± 1.4	7.4 ± 0.5	19.9 ± 4.2
Brain	0.5 ± 0.1	1.5 ± 0.1	5.1 ± 1.7
Lungs	2.8 ± 1.3	6.1 ± 3.2	12.5 ± 3.2
Skeletal Muscle	0.5 ± 0.1	1.8 ± 0.2	5.4 ± 1.9

a/ Radioactivity in 1 ml or gm of wet tissue per radioactivity in 1 ml of plasma.

b/ Mean ± S.E. of three rats.

TABLE 11

ACCUMULATION OF RADIOACTIVITY IN RATS RECEIVING 2,4-DNT (RING-UL-^{14}C)

Tissue	^{14}C 24 Hours After A Single Dose (dpm/gm)[a/]	^{14}C 24 Hours After the Last of Five Daily Doses (dpm/gm)[a/]	Ratio[b/]
Blood	968	4,483	4.6
Liver	6,804	22,777	3.3
Kidney	2,728	13,216	4.8
Brain	558	1,133	2.0
Lungs	1,118	4,540	2.5
Skeletal Muscle	653	2,105	3.3

a/ Average of three rats.

b/ Radioactivity per gm of wet tissue from rats receiving five consecutive daily doses assayed 24 hours after the last dose per radioactivity per gm of wet tissue from rats receiving a single dose assayed 24 hours after dosing.

TABLE 12

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING A SINGLE DOSE OF 2,5-DNT (RING-UL-^{14}C)

	% of Administered Dose		Tissue/Plasma Radioactivity Ratio[d]	
	4 Hours	24 Hours	4 Hours	24 Hours
Gastrointestinal tract plus contents	85.5 ± 4.9[c]	1.9 ± 0.5		
Feces	< 0.1	29.1 ± 5.9		
Whole Blood[a]	0.3 ± 0.1	< 0.1		
Urine	13.5 ± 3.1	60.5 ± 6.5		
Expired Air	-	< 0.1		
Liver	0.9 ± 0.1	0.9 ± 0.1	4.7 ± 1.5	29.1 ± 5.5
Kidneys	0.3 ± 0.1	< 0.1	5.9 ± 0.5	9.7 ± 1.7
Spleen	< 0.1	< 0.1	1.9 ± 1.0	1.1 ± 0.1
Brain	< 0.1	< 0.1	0.5 ± 0.0	0.5 ± 0.1
Lungs	< 0.1	< 0.1	1.1 ± 0.3	2.7 ± 0.7
Skeletal Muscle[b]	1.1 ± 0.5	0.5 ± 0.3	0.5 ± 0.1	0.9 ± 0.1
Recovery	101.7% ± 1.7	92.9% ± 2.1		

a/ Based on 7% of the body weight.
b/ Based on 40% of the body weight.
c/ Mean ± S.E. of three rats.
d/ Radioactivity in 1 ml or gm of wet tissue per radioactivity in 1 ml of plasma.

TABLE 13

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING A SINGLE DOSE OF 2,6-DNT (RING-UL-^{14}C)

	% of Administered Dose		Tissue/Plasma Radioactivity Ratio[e]	
	4 Hours	24 Hours	4 Hours	24 Hours
Gastrointestinal tract plus contents	83.5 ± 8.6[c,d]	10.5 ± 3.5		
Feces	-	27.5 ± 7.0		
Whole Blood[a]	0.4 ± 0.1	0.3 ± 0.0		
Expired Air	-	0.1 ± 0.0		
Urine	10.4 ± 3.6	60.4 ± 2.6		
Liver	0.8 ± 0.1	0.9 ± 0.3	4.7 ± 1.2	11.4 ± 3.7
Kidneys	0.2 ± 0.1	0.1 ± 0.0	4.5 ± 0.5	7.3 ± 1.0
Spleen	< 0.1	< 0.1	1.4 ± 0.2	2.4 ± 0.6
Brain	< 0.1	< 0.1	0.9 ± 0.1	0.9 ± 0.1
Lungs	< 0.1	< 0.1	1.2 ± 0.1	2.7 ± 0.5
Skeletal Muscle[b]	2.5 ± 5.0	0.9 + 0.1	1.0 ± 0.1	1.1 ± 0.2
Recovery	98.0 ± 5.0	100.6 ± 5.1		

a/ Based on 7% of the body weight.
b/ Based on 40% of the body weight.
c/ Mean ± S.E. of three rats.
d/ Feces and G.I. tract plus contents were pooled for the 4-hour experiment.
e/ Radioactivity in 1 ml or gm of wet tissue per radioactivity in 1 ml of plasma.

TABLE 14

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING A SINGLE DOSE OF 3,4-DNT (RING-UL-^{14}C)

	% of Administered Dose		Tissue/Plasma Radioactivity Ratio[d]	
	30 Minutes[c]	24 Hours	30 Minutes	24 Hours
Gastrointestinal tract plus contents	-	2.6 ± 1.0	-	-
Feces	-	12.1 ± 6.0	-	-
Whole Blood[a]	0.5 ± 0.2	< 0.1	-	-
Expired Air	-	< 0.1	-	-
Urine	-	87.4 ± 8.9	-	-
Liver	0.6 ± 0.1	< 0.1	1.7 ± 0.3	6.2 ± 0.9
Kidneys	0.3 ± 0.0	< 0.1	2.8 + 0.6	4.7 ± 1.0
Brain	< 0.1	< 0.1	0.5 ± 0.0	0.6 ± 0.0
Lungs	-	< 0.1	-	1.7 ± 0.3
Skeletal Muscle[b]	0.9 ± 0.3	0.2 ± 0.0	0.2 ± 0.0	1.0 ± 0.1
Recovery	-	102.0 ± 2.0		

a/ Based on 7% of the body weight.
b/ Based on 40% of the body weight.
c/ Mean ± S.E. of three rats.
d/ Radioactivity in 1 ml or gm of wet tissue per radioactivity of 1 ml of plasma.

TABLE 15

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING A SINGLE DOSE OF TNG-1,3-^{14}C

	% of Administered Dose		Tissue/Plasma Radioactivity Ratio [f]	
	4 Hours	24 Hours	4 Hours	24 Hours
Gastrointestinal tract plus contents	59.5 ± 13.6 [d]	3.0 ± 0.2 [e]	-	-
Feces	3.1 ± 1.9	6.3 ± 1.0	-	-
Whole Blood [a]	2.1 ± 0.6	0.7 ± 0.0	-	-
Expired Air	7.5 ± 1.2	25.5 ± 1.5	-	-
Urine	15.6 ± 4.7	39.8 ± 2.3	-	-
Liver	4.6 ± 0.4	4.3 ± 0.9	4.4 ± 2.0	7.8 ± 1.7
Kidneys	0.7 ± 0.1	0.3 ± 0.0	2.4 ± 0.5	2.8 ± 0.3
Brain	0.3 ± 0.1	0.1 ± 0.0	1.1 ± 0.1	1.0 ± 0.1
Lungs	0.2 ± 0.1	0.1 ± 0.0	1.2 ± 0.1	1.7 ± 0.1
Skeletal Muscle [b]	9.3 ± 2.6	2.8 ± 0.2	0.6 ± 0.1	0.6 ± 0.1
Carcass [c]	-	5.4 ± 0.6		
Recovery	102.9 ± 10.0	87.3 ± 7.0		

a/ Based on 7% of the body weight.
b/ Based on 40% of the body weight.
c/ Corrected for radioactivity in muscle.
d/ Mean ± S.E. of three rats.
e/ Mean ± S.E. of four rats.
f/ Radioactivity in 1 ml or gm of wet tissue per radioactivity in 1 ml of plasma.

TABLE 16

METABOLITES OF TNG IN RAT URINE 24 HOURS AFTER ORAL ADMINISTRATION OF TNG-1,3-^{14}C

Metabolite	% of Administered Dose
TNG	< 0.1
1,2-DNG	0.7 ± 0.4[b]
1,3-DNG	0.4 ± 0.2
MNGs	10.6 ± 1.3
1,2-DNG-glucuronide	10.0 ± 0.7
1,3-DNG-glucuronide	3.5 ± 0.4
MNG-glucuronides	1.5 ± 0.2
Glycerol	6.9 ± 0.8
Others[a]	
R_f = 0.0	4.9 ± 1.5
R_f = 0.6	1.3 ± 0.3

a/ Unidentified polar components with n-butanol: acetic acid:water solvent system.

b/ Mean ± S.E. of three cats.

TABLE 17

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING A SINGLE DOSE OF 1,2-DNG-1,3-^{14}C

	% of Administered Dose		Tissue/Plasma Radioactivity Ratio[e]	
	4 Hours	24 Hours	4 Hours	24 Hours
Gastrointestinal tract plus contents	51.9 ± 6.0[d]	2.5 ± 0.3	-	-
Feces	4.5 ± 2.9	11.7 ± 4.7	-	-
Whole Blood[a]	2.4 ± 0.2	0.5 ± 0.0	-	-
Expired Air	5.7 ± 0.5	20.0 ± 2.5	-	-
Urine	21.1 ± 3.7	50.5 ± 7.5	-	-
Liver	3.8 ± 0.9	3.8 ± 0.6	3.3 ± 0.7	10.6 ± 1.4
Kidneys	0.4 ± 0.1	0.2 ± 0.0	1.7 ± 0.1	3.6 ± 0.3
Spleen	0.1 ± 0.0	< 0.1	1.0 ± 0.1	2.4 ± 0.1
Brain	0.3 ± 0.0	< 0.1	0.9 ± 0.1	0.8 ± 0.1
Lungs	0.2 ± 0.0	0.1 ± 0.0	1.0 ± 0.1	1.9 ± 0.1
Skeletal Muscle[b]	7.7 ± 0.6	1.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.0
Carcass[c]	-	5.3 ± 0.7		
Recovery	97.8 ± 3.2	96.3 ± 2.4		

a/ Based on 7% of the body weight.
b/ Based on 40% of the body weight.
c/ Corrected for radioactivity in muscle.
d/ Mean ± S.E. of three rats.
e/ Radioactivity in 1 ml or gm of wet tissue per radioactivity in 1 ml of plasma.

TABLE 18

METABOLITES OF DNG IN RAT URINE 24 HOURS AFTER ORAL ADMINISTRATION OF 1,2-DNG-1,3-^{14}C

Metabolite	% of Administered Dose
1,2-DNG	1.0 ± 0.8[b]
MNGs	15.4 ± 0.4
1,2-DNG-glucuronide	15.3 ± 0.8
MNG-glucuronides	3.2 ± 0.2
Glycerol	8.7 ± 1.0
Others[a]	
R_f = 0.0	6.2 ± 1.0
R_f = 0.6	0.7 ± 0.1

a/ Unidentified polar components with n-butanol: acetic acid:water solvent system.

b/ Mean ± S.E. of three rats.

TABLE 19

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING A SINGLE DOSE OF 1,3-DNG-1,3-^{14}C

	% of Administered Dose		Tissue/Plasma Radioactivity Ratio[f]	
	4 Hours	24 Hours	4 Hours	24 Hours
Gastrointestinal tract plus contents	65.4 ± 1.5[d]	4.3 ± 0.2	-	-
Feces	1.1 ± 0.7[e]	11.6 ± 5.3	-	-
Whole Blood[a]	1.8 ± 0.2	0.9 ± 0.0	-	-
Expired Air	7.5 ± 0.1	25.1 ± 0.7	-	-
Urine	6.9 ± 0.5	27.7 ± 9.8	-	-
Liver	9.3 ± 1.9	7.1 ± 0.5	9.1 ± 1.5	14.5 ± 2.7
Kidneys	0.5 ± 0.0	0.3 ± 0.0	2.1 ± 0.4	4.9 ± 1.5
Spleen	0.1 ± 0.0	0.1 ± 0.0	1.0 ± 0.2	2.5 ± 0.7
Brain	0.3 ± 0.0	0.1 ± 0.0	1.3 ± 0.2	1.4 ± 0.4
Lungs	0.2 ± 0.0	0.1 ± 0.0	1.1 ± 0.2	2.5 ± 0.7
Skeletal Muscle[b]	6.2 ± 0.8	4.5 ± 0.0	0.5 ± 0.0	1.1 ± 0.4
Carcass[c]	-	7.9 ± 0.4		
Recovery	99.0 ± 0.2	90.0 ± 4.3		

a/ Based on 7% of the body weight.
b/ Based on 40% of the body weight.
c/ Corrected for radioactivity in muscle.
d/ Mean ± S.E. of three rats.
e/ Mean of two rats.
f/ Radioactivity in 1 ml or gm of wet tissue per radioactivity in 1 ml of plasma.

TABLE 20

METABOLITES OF DNG IN RAT URINE 24 HOURS AFTER ORAL ADMINISTRATION OF 1,3-DNG-1,3-^{14}C

Metabolite	% of Administered Dose
1,3-DNG	0.2 ± 0.0[b]
1-MNG	5.0 ± 0.2
1,3-DNG-glucuronide	4.0 ± 0.8
1-MNG-glucuronide	1.1 ± 0.8
Glycerol	9.2 ± 0.5
Others[a]	
R_f = 0.0	6.9 ± 0.7
R_f = 0.6	1.3 ± 0.2

a/ Unidentified polar components with n-butanol: acetic acid:water solvent system.

b/ Mean ± S.E. of three rats.

TABLE 21

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING A SINGLE DOSE OF 1-MNG-1,3-^{14}C

	% of Administered Dose		Tissue/Plasma Radioactivity Ratio[e]	
	4 Hours	24 Hours	4 Hours	24 Hours
Gastrointestinal Tract Plus Contents	33.8 ± 5.6[c,d]	3.2 ± 1.0	-	-
Feces	-	14.4 ± 6.1	-	-
Whole Blood[a]	4.1 ± 0.4	0.9 ± 0.1	-	-
Expired Air	9.2 ± 0.8	30.5 ± 4.6	-	-
Urine	18.9 ± 2.0	29.0 ± 10.4	-	-
Liver	4.2 ± 0.2	2.9 ± 1.1	2.0 ± 0.3	2.6 ± 0.3
Kidneys	1.0 ± 0.1	0.4 ± 0.1	1.9 ± 0.13	3.5 ± 0.9
Spleen	0.1 ± 0.0	< 0.1	1.0 ± 0.1	1.7
Brain	0.6 ± 0.1	0.1 ± 0.0	1.1 ± 0.1	1.0 ± 0.1
Lungs	0.3 ± 0.0	0.1 ± 0.0	0.8 ± 0.0	1.3 ± 0.2
Skeletal Muscle[b]	15.0 ± 1.6	3.0 ± 0.4	0.6 ± 0.0	0.4 ± 0.0
Carcass	10.8 ± 1.4	6.4 ± 0.7	-	-
Recovery	96.4 ± 2.2	90.9 ± 3.3	-	-

a/ Based on 7% of the body weight.
b/ Based on 40% of the body weight.
c/ Mean ± S.E. of four rats.
d/ Feces of G.I. tract plus contents were pooled for the 4-hour experiment.
e/ Radioactivity in 1 ml or gm of wet tissue per radioactivity in 1 ml of plasma.

TABLE 22

METABOLITES OF 1-MNG IN RAT URINE 24 HOURS AFTER ORAL ADMINISTRATION OF 1-MNG-1,3-^{14}C

Metabolite	% of Radioactivity in Urine
1-MNG	13.3 ± 1.0[b]
Glycerol	10.1 ± 0.5
Other[a] R_f = 0.0	5.6 ± 0.5

a/ Unidentified polar components with n-butanol: acetic acid:water solvent system.

b/ Mean ± S.E. of three rats.

TABLE 23

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING A SINGLE DOSE OF 2-MNG-1,3-^{14}C

	% of Administered Dose		Tissue/Plasma Radioactivity Ratio[c]	
	4 Hours	24 Hours	4 Hours	24 Hours
Gastrointestinal Tract Plus Contents	50.1 ± 10.7[c]	1.2 ± 0.3	-	-
Feces	3.9 ± 1.9	32.1 ± 15.1	-	-
Whole Blood[a]	2.5 ± 0.4	0.3 ± 0.1	-	-
Expired Air	1.6 ± 0.3	7.2 ± 1.1	-	-
Urine	28.3 ± 9.3	48.8 ± 16.4	-	-
Liver	1.9 ± 0.5	1.4 ± 0.2	1.5 ± 0.2	14.4 ± 4.1
Kidneys	0.4 ± 0.0	< 0.1	1.4 ± 0.0	3.2 ± 0.4
Spleen	0.1 ± 0.0	< 0.1	1.0 ± 0.0	2.7 ± 0.5
Brain	0.3 ± 0.0	< 0.1	0.8 ± 0.0	1.2 ± 0.2
Lungs	0.2 ± 0.0	< 0.1	1.0 ± 0.0	2.5 ±0.4
Skeletal Muscle[b]	10.6 ± 1.0	1.1 ± 0.2	0.7 ± 0.0	1.2 ± 0.3
Carcass	5.1 ± 2.3	1.0 ± 0.2	-	-
Recovery	104.8 ± 7.6	92.2 ± 9.8		

a/ Based on 7% of the body weight.
b/ Based on 40% of the body weight.
c/ Mean ± S.E. of three rats.
d/ Radioactivity in 1 ml or gm of wet tissue per radioactivity in 1 ml of plasma.

TABLE 24

METABOLITES OF 2-MNG IN RAT URINE 24 HOURS AFTER ORAL ADMINISTRATION OF 2-MNG-1,3-^{14}C

Metabolite	% of Administered Dose
2-MNG	24.4 ± 2.6[b]
Glycerol	21.7 ± 3.7
Other[a] R_f = 0.0	2.7 ± 1.6

[a] Unidentified polar components with n-butanol: acetic acid:water solvent system.

[b] Mean ± S.E. of three rats.

TABLE 25

DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING ^{32}P WHITE PHOSPHORUS

	% of Administered Dose		
	4 Hours	1 Day	5 Days
Gastrointestinal Tract Plus Contents	57.0 ± 3.4[c]	15.3 ± 4.0	1.7 ± 0.2
Feces	2.0 ± 1.0	16.6 ± 3.8	33.0[d]
Whole Blood[a]	6.1 ± 1.1	4.1 ± 0.5	1.7 ± 0.0
Urine	17.1 ± 2.2	34.5 ± 6.1	46.7[d]
Liver	16.1 ± 4.6	16.9 ± 0.7	6.3 ± 0.3
Kidneys	0.7 ± 0.2	0.8 ± 0.1	0.4 ± 0.0
Spleen	0.1 ± 0.0	0.1 ± 0.0	0.1
Brain	0.1 ± 0.0	0.1	0.1
Lungs	0.4 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
Skeletal Muscle[b]	4.0 ± 0.0	5.5 ± 0.2	6.0 ± 0.6
Recovery	98.6 ± 5.0	94.0 ± 3.3	96.0

a/ Based on 7.0% of the body weight.
b/ Based on 40% of the body weight.
c/ Mean ± S.E. of three rats.
d/ Pooled samples from three rats.

TABLE 26

TISSUE/PLASMA RATIOS OF RADIOACTIVITY IN RATS RECEIVING A SINGLE DOSE OF ^{32}P WHITE PHOSPHORUS

Tissue	Tissue/Plasma Radioactivity Ratio[a] 4 Hours	1 Day	5 Days
Liver	18.7 ± 2.5[b]	51.4 ± 3.9	103.2 ± 10.0
Kidneys	4.2 ± 1.0	14.4 ± 1.2	33.5 ± 4.1
Spleen	1.8 ± 0.4	6.4 ± 2.6	18.6 ± 2.6
Brain	0.3 ± 0.0	0.7 ± 0.0	3.6 ± 0.4
Lungs	2.6 ± 0.1	5.8 ± 0.5	16.5 ± 1.0
Skeletal Muscle	0.4 ± 0.0	1.8 ± 0.1	8.7 ± 0.5
Bone	1.7 ± 0.1	12.7 ± 0.1	66.9 ± 17.2

a/ Radioactivity in 1 ml or gm of wet tissue per radioactiviy in 1 ml or plasma.

b/ Mean ± S.E. of three rats.

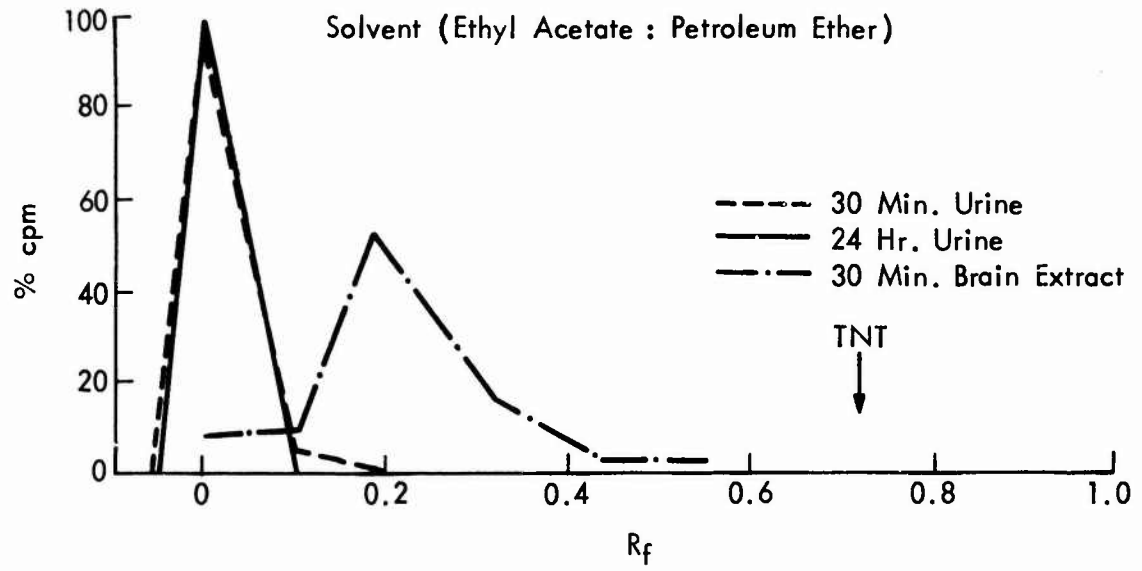
TABLE 27

ACCUMULATION OF RADIOACTIVITY IN RATS RECEIVING ^{32}P WHITE PHOSPHORUS

	^{32}P 24 Hours After A Single Dose (dpm/gm x 10^{-5})	^{32}P 24 Hours After the Last of Five Daily Doses (dpm/gm x 10^{-5})	Ratio[b]
Blood	2.91[a]	28.42	9.8
Liver	19.30	79.44	4.1
Kidney	5.44	39.29	7.2
Spleen	2.38	17.70	7.4
Brain	0.25	2.63	10.5
Lungs	2.18	22.21	10.2
Skeletal Muscle	0.68	5.90	8.7
Bone	4.77	36.81	7.7

a/ Average of three rats.

b/ Radioactivity per gm of wet tissue from rats receiving five consecutive daily doses assayed 24 hours after the last dose per radioactivity per gm of wet tissue from rats receiving a single dose assayed 24 hours after dosing.



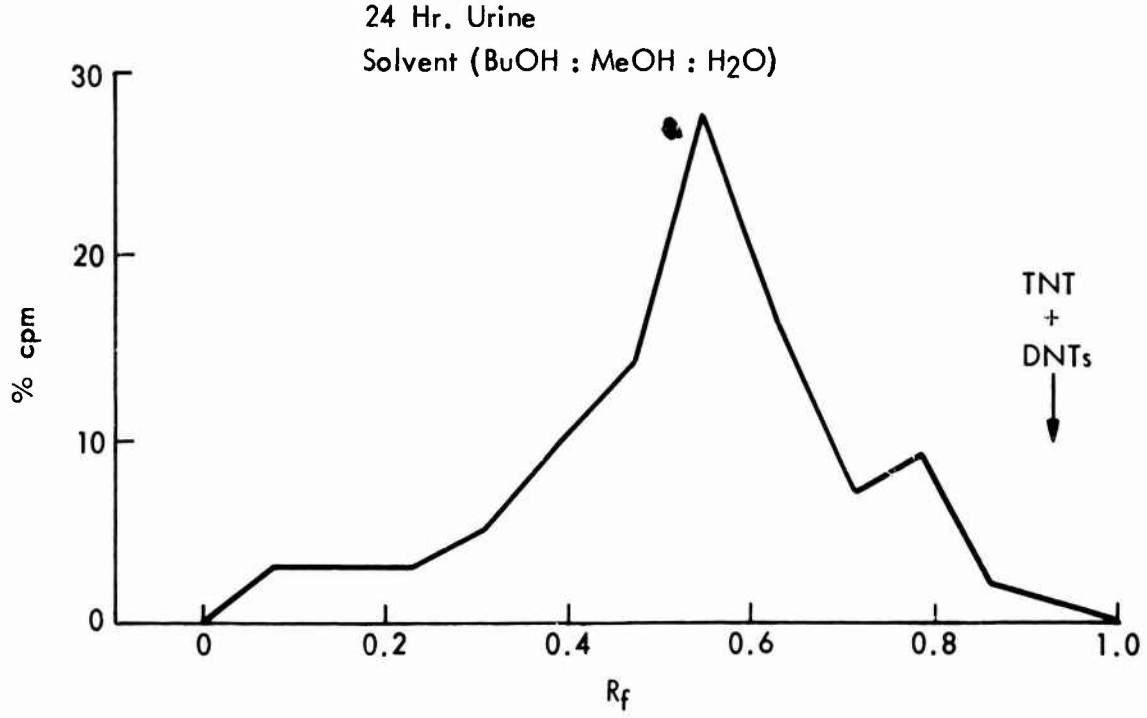
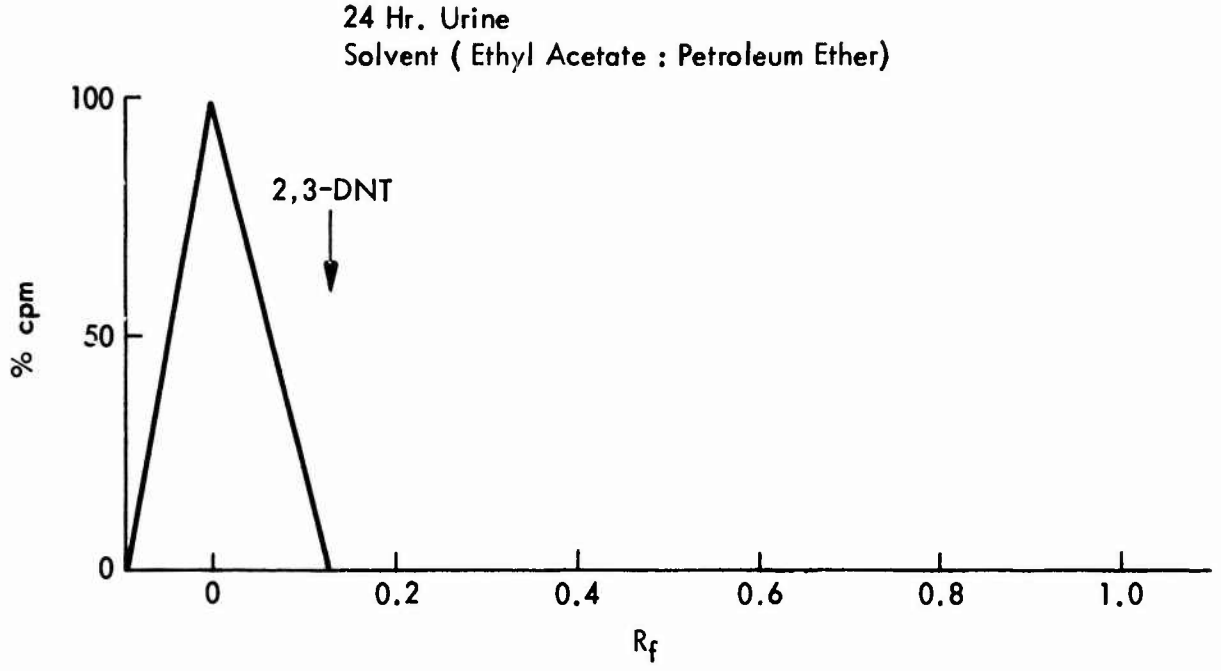


Figure 1 - TLC of Rat Urine or Brain Extract After Oral Administration of TNT (Ring-UL-^{14}C)



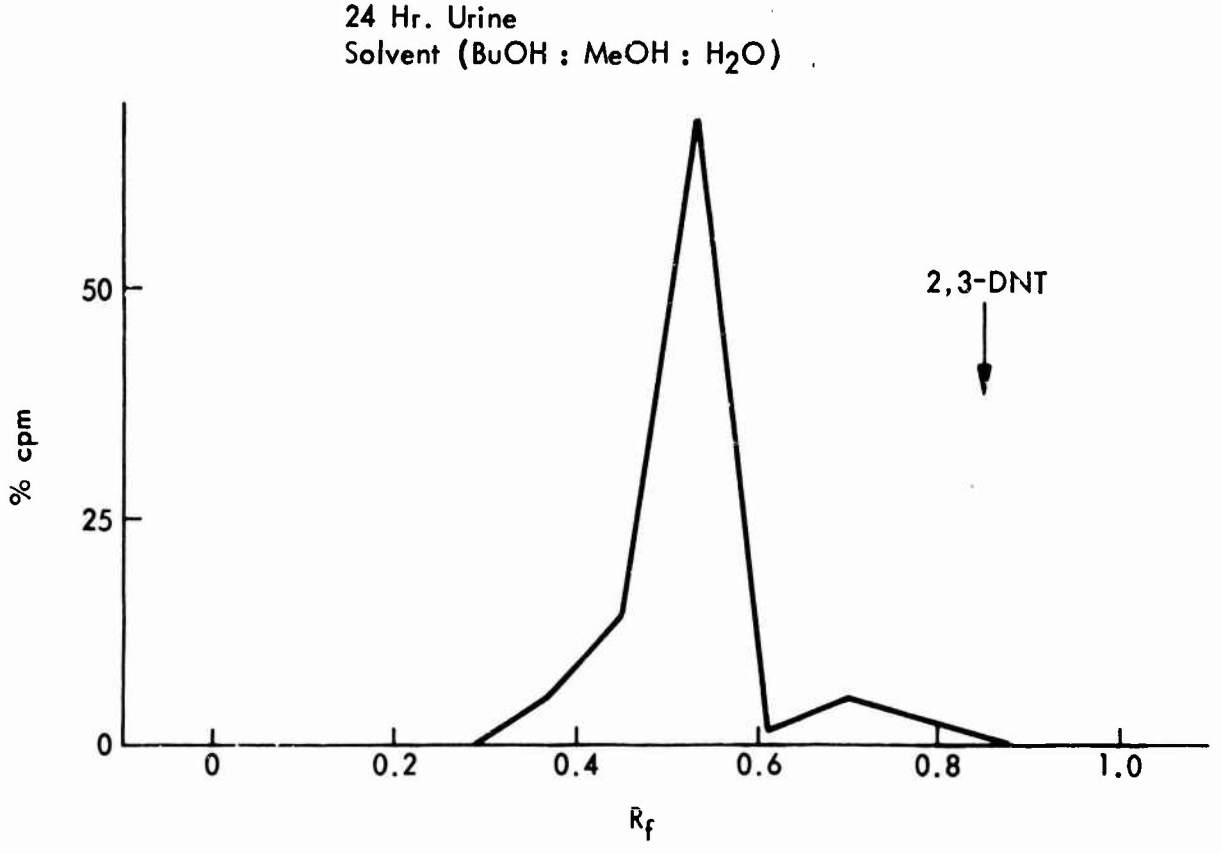
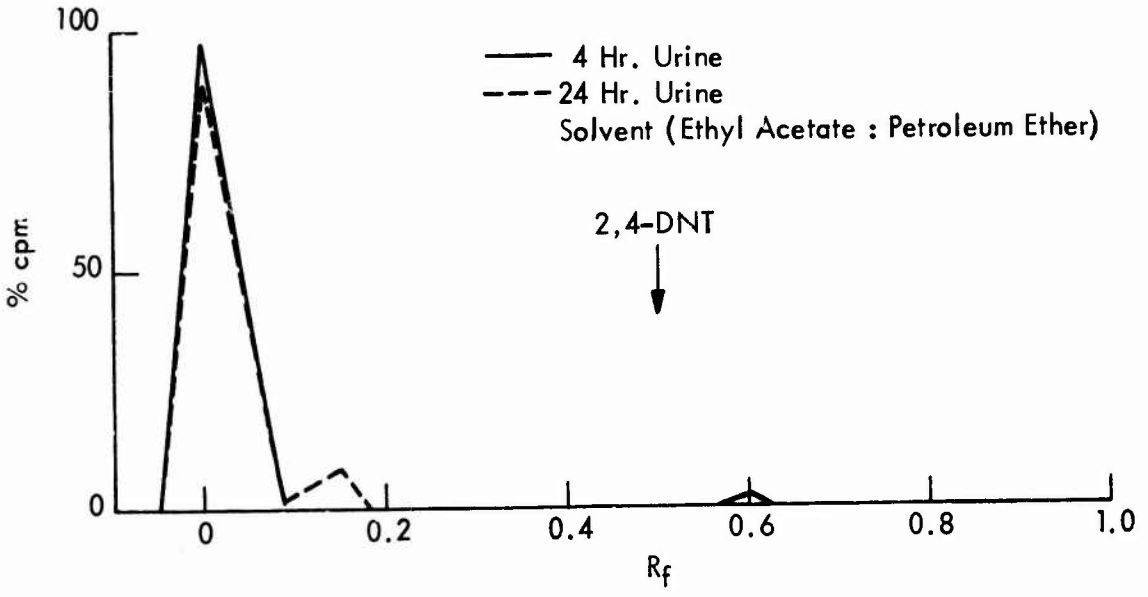


Figure 2 - TLC of Rat Urine After Oral Administration of 2,3-DNT (Ring-UL-^{14}C)



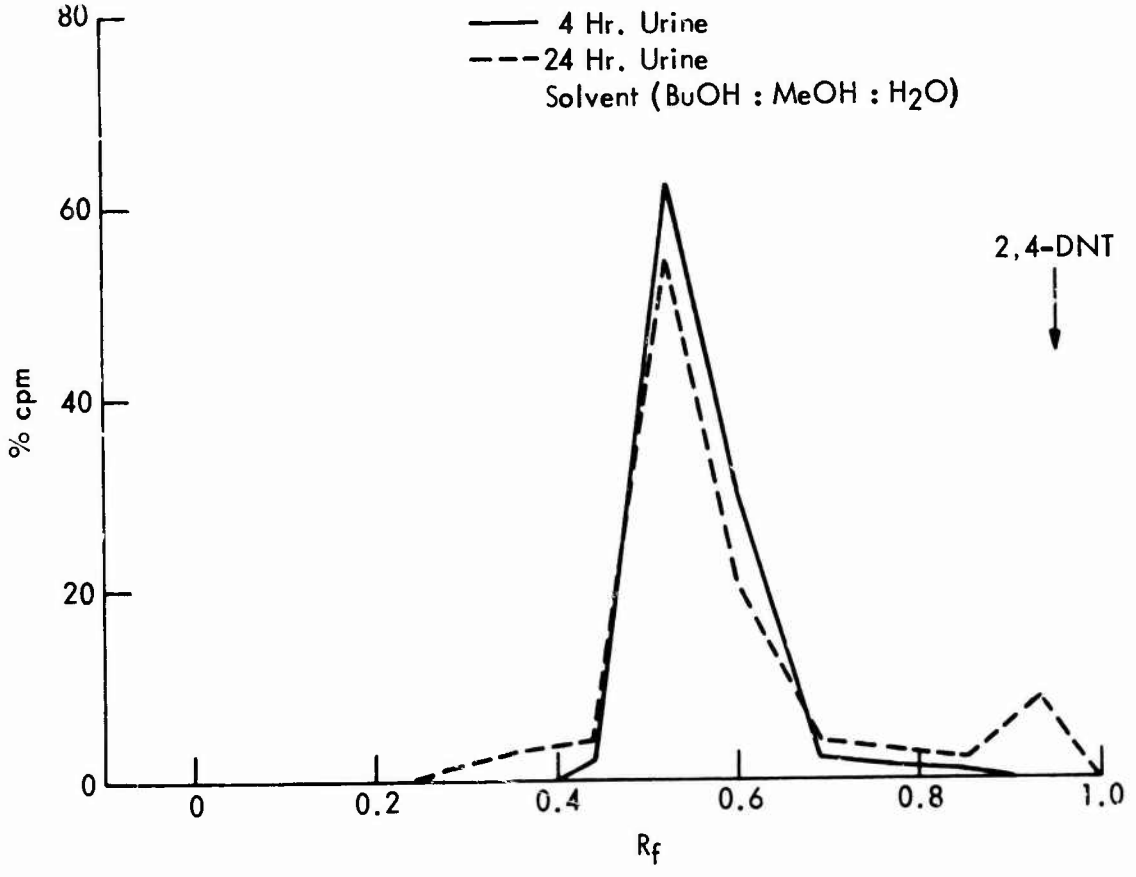
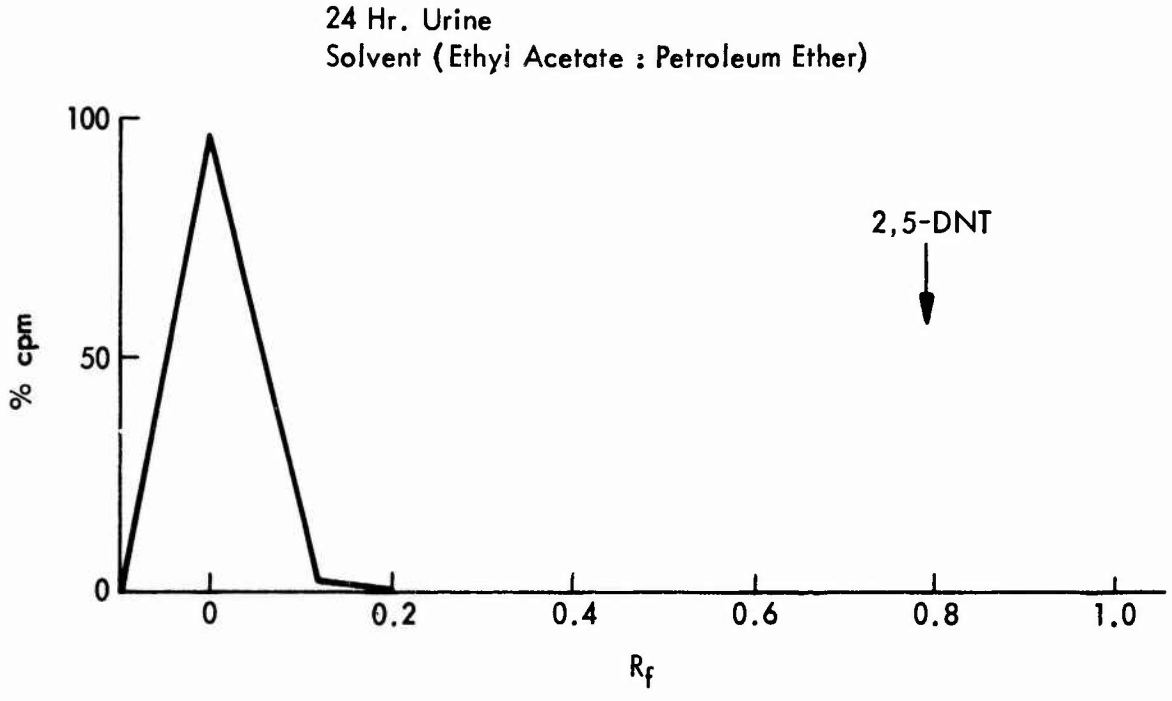


Figure 3 - TLC of Rat Urine After Oral Administration of 2,4-DNT (Ring-UL-^{14}C)



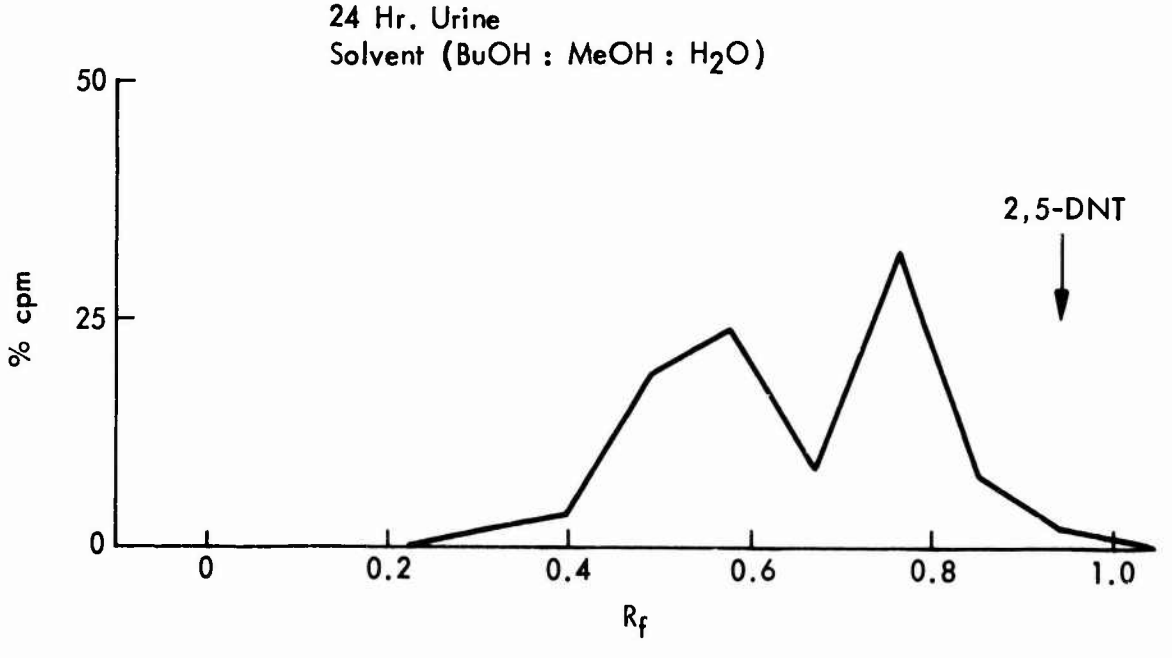
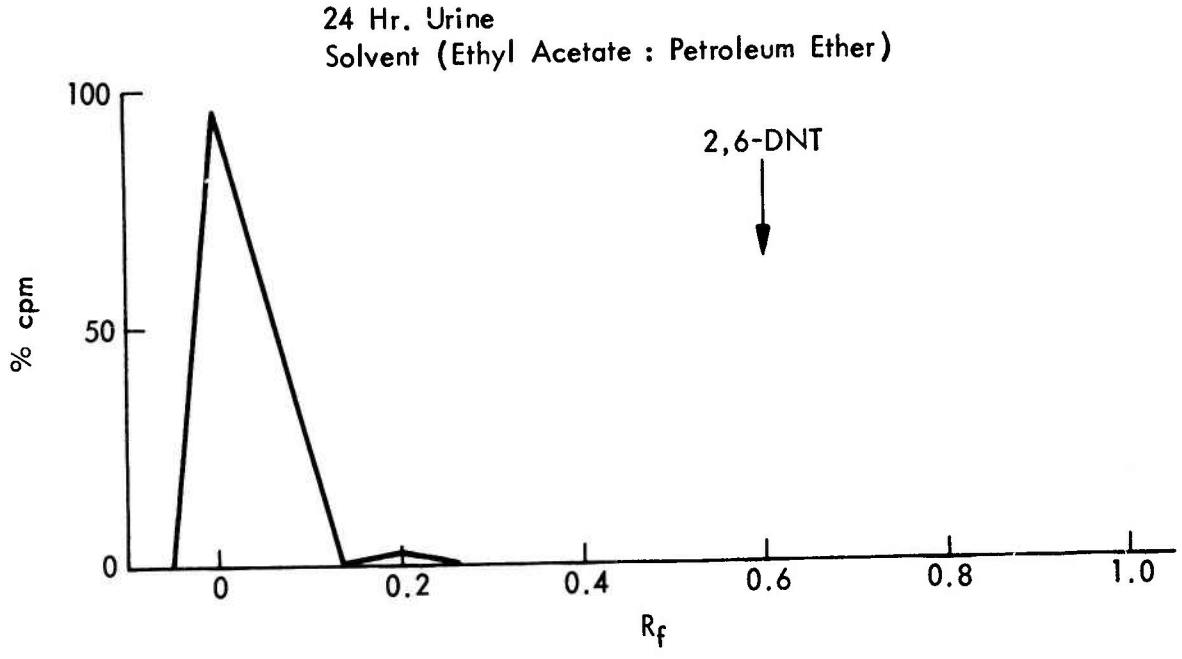


Figure 4 - TLC of Rat Urine After Oral Administration of 2,5-DNT (Ring-UL-^{14}C)



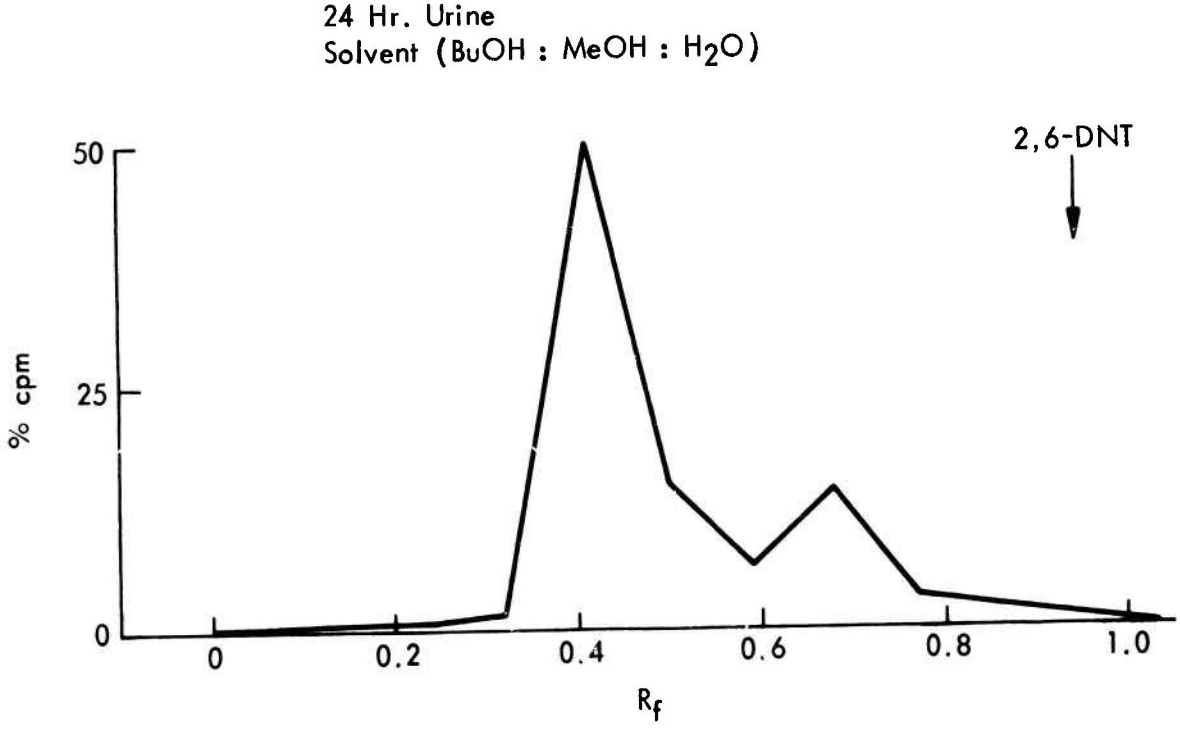


Figure 5 - TLC of Rat Urine After Oral Administration of 2,6-DNT (Ring-UL-^{14}C)

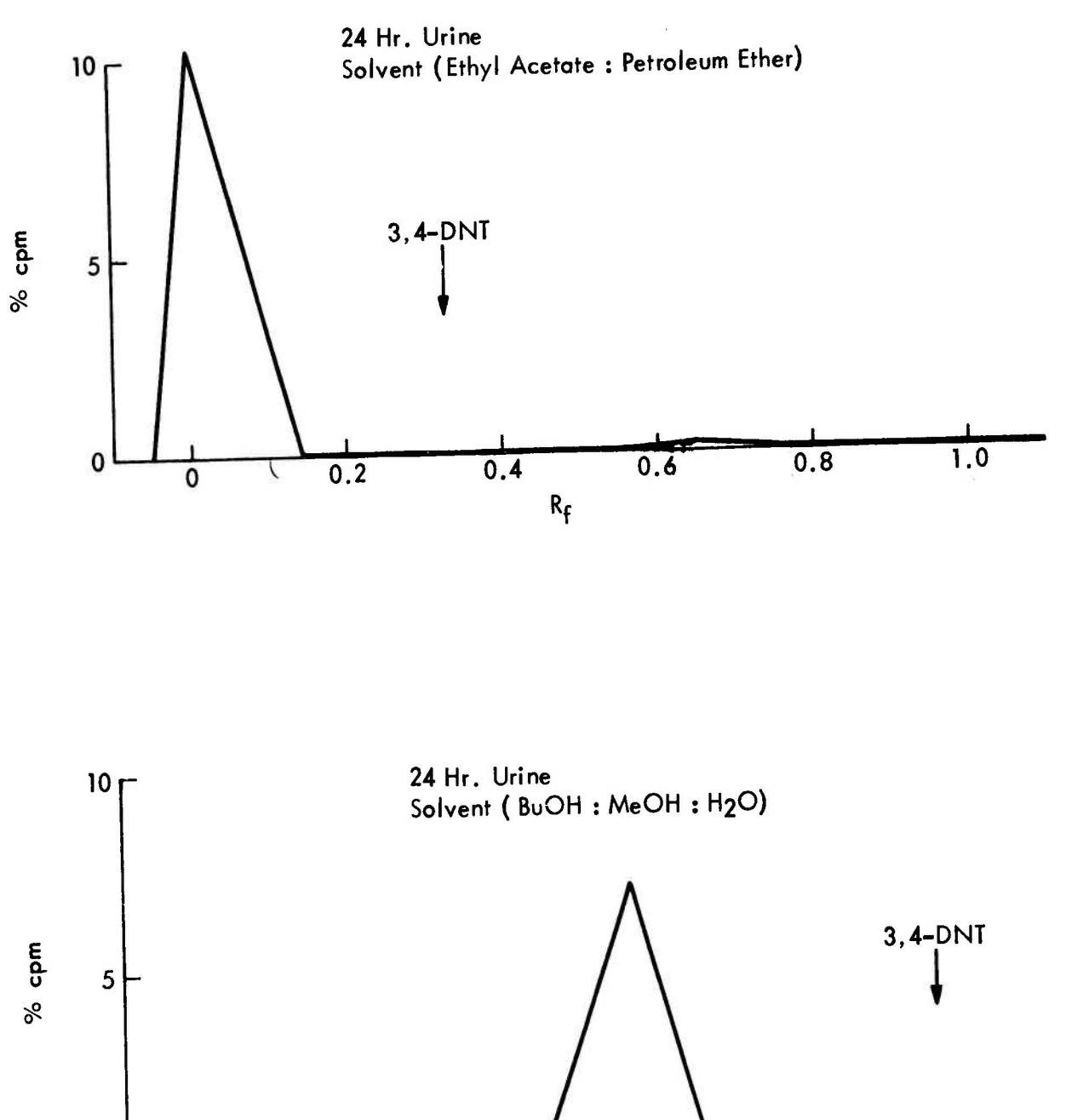
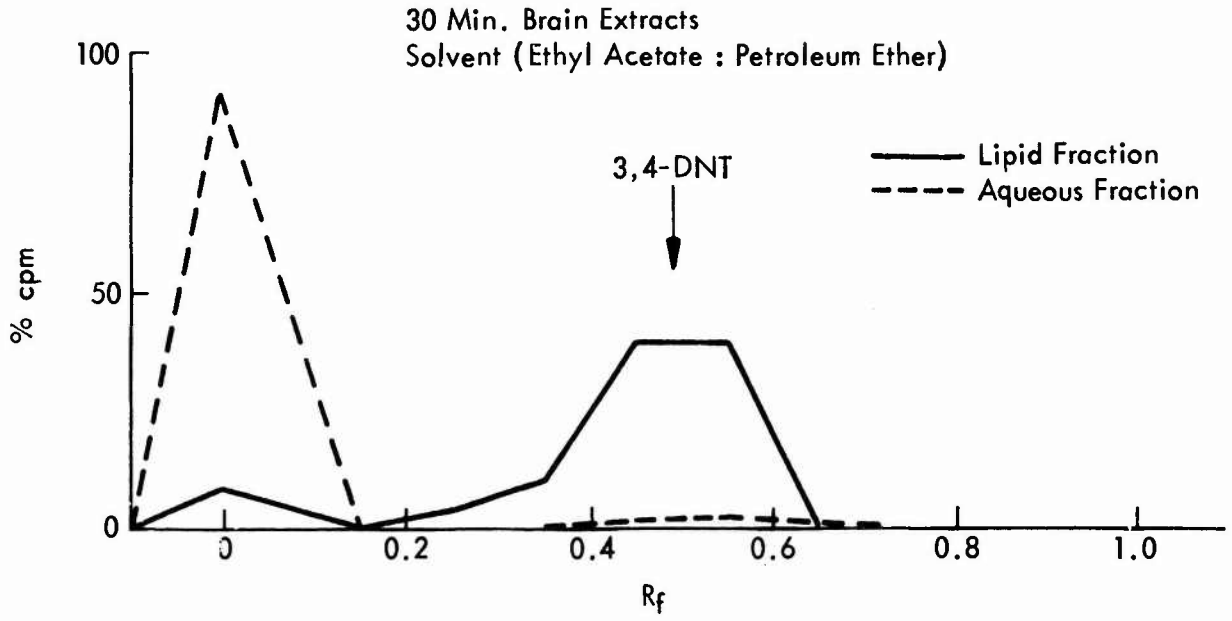


Figure 6 - TLC of Rat Urine After Oral Administration of 3,4-DNT (Ring-UL-^{14}C)



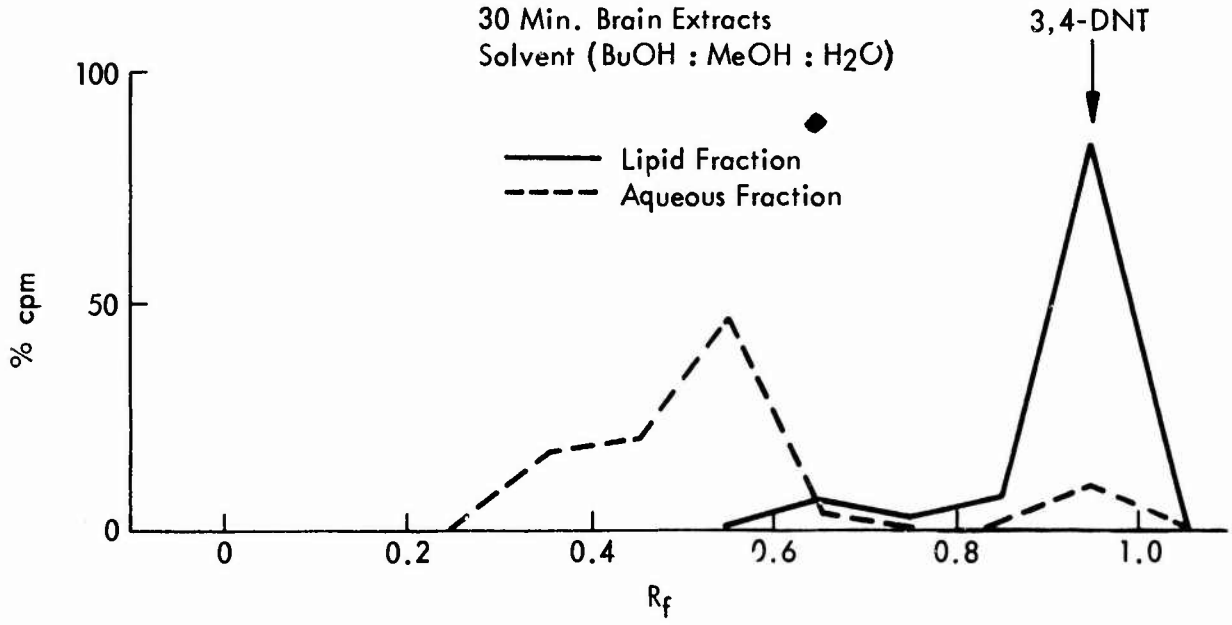


Figure 7 - TLC of a Lipid and Aqueous Extract of Rat Brain After Oral Administration of 3,4-DNT (King-UL-^{14}C)

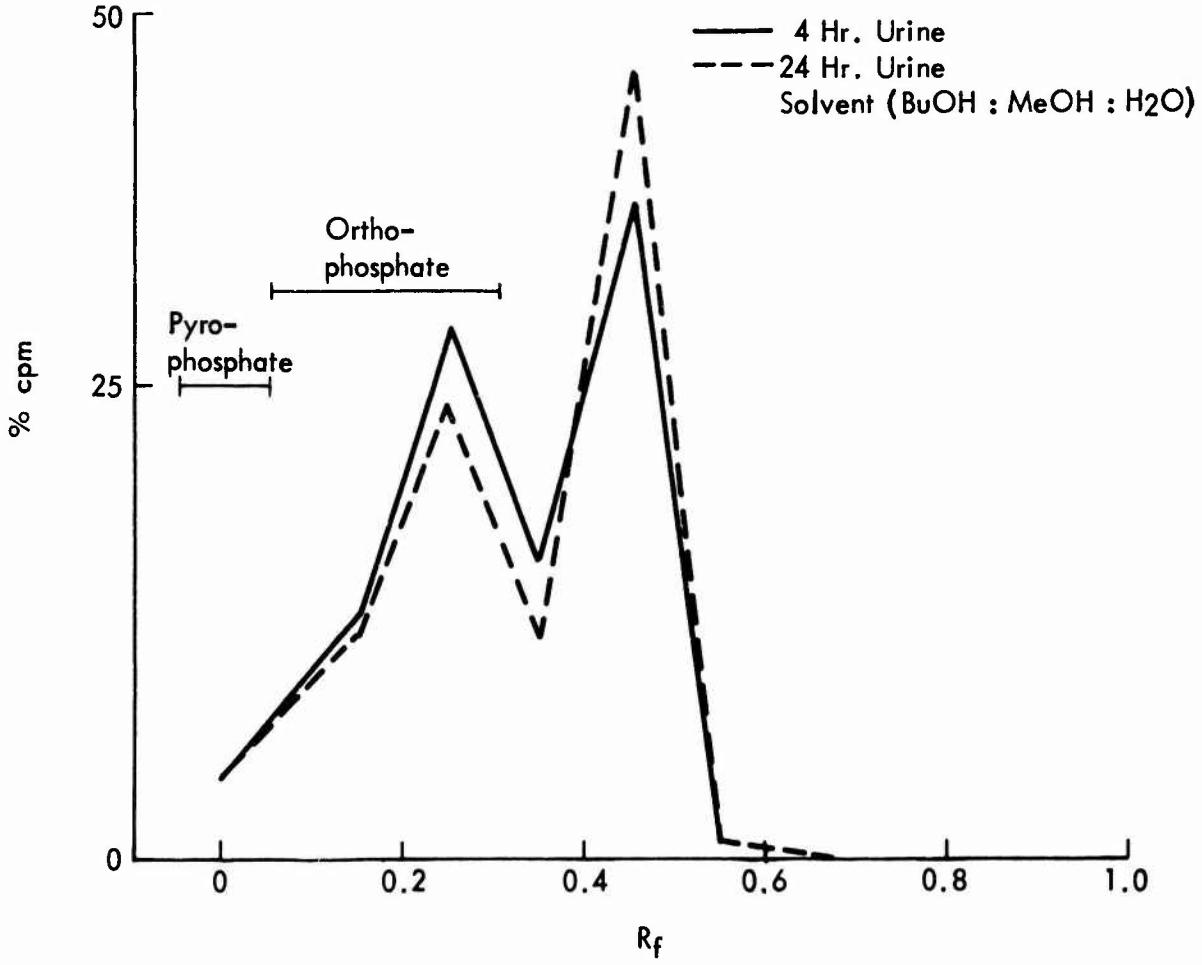


Figure 8 - TLC of Rat Urine After Oral Administration of White Phosphorus (^{32}P)

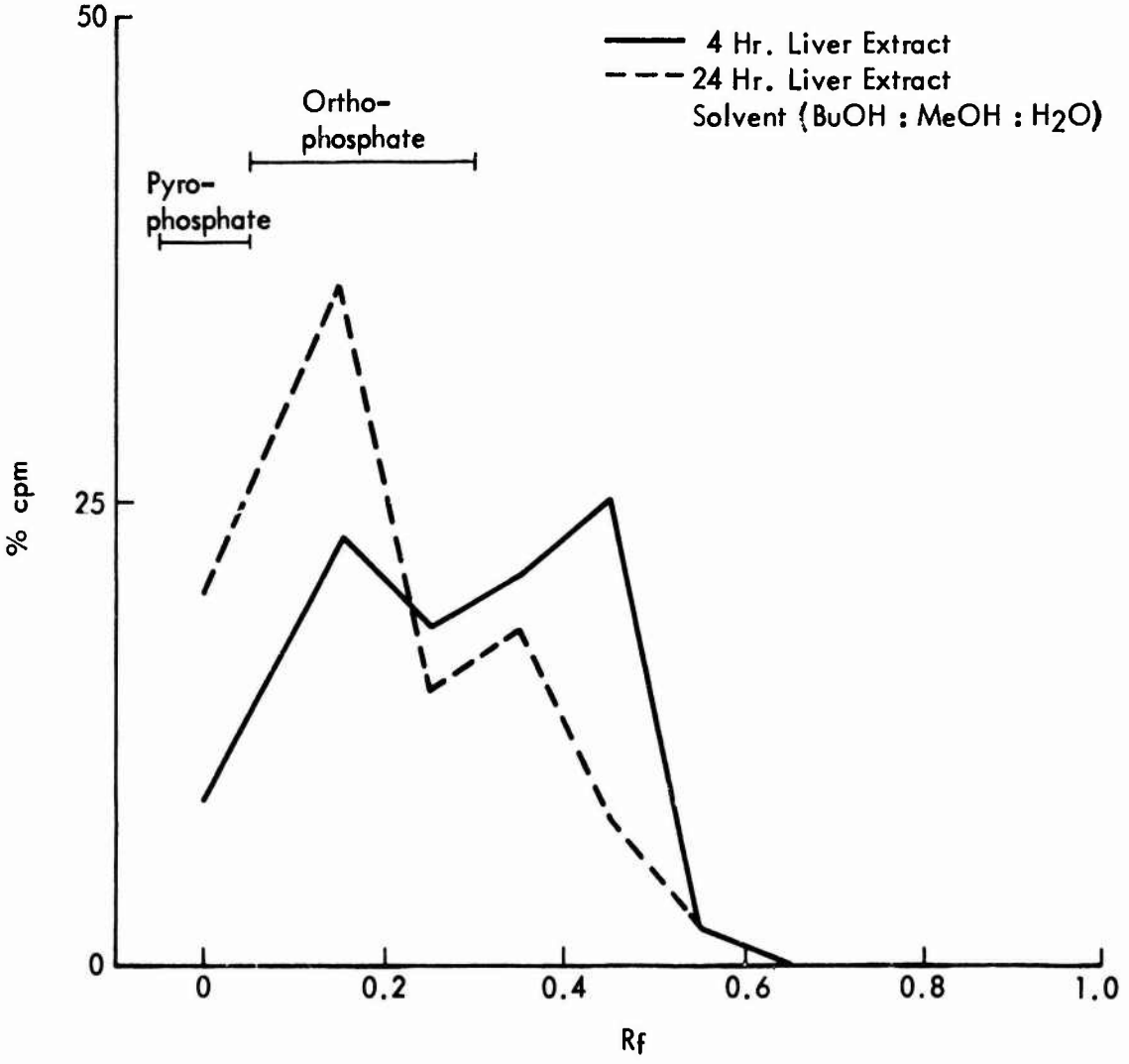


Figure 9 - TLC of Rat Liver Extract After Oral Administration of White Phosphorus (^{32}P)

REFERENCES

1. Dunstan, I., J. V. Griffiths, and S. A. Harvey, Nitric Esters, Part I. Characterization of the Isomeric Glycerol Dinitrates, J. Chem. Soc., :1319 (1965).

2. Nichols, P. L., A. B. Magnusson, and J. D. Ingham, J. Am. Chem. Soc., 75:4255 (1953).

3. Selig, W., Microdetermination of Aromatic Nitro Compounds, Nitrocellulose and Cyclic Nitramines, AEC Report UCRL-6639.

4. Finney, D. J., Probit Analysis, Cambridge University Press (1971).

5. Federal Register, Title 21 - Food and Drugs, Part 191.11 "Method of Testing Primary Irritant Substances" and Part 191.12 "Test for Eye Irritants" (1974).

6. Magnusson, B. and Albert M. Kligman, The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test, J. Invest. Derm., 52:268-276 (1969).

7. Roth, L. J., E. Leiter, J. R. Hogness, and W. H. Langham, Studies on the Metabolism of Radioactive Nicotinic Acid and Nicotinamide in Mice, J. Biol. Chem., 176:249-257 (1948).

8. DiCarlo, F. J., Malcolm C. Crew, Lloyd J. Haynes, Myriam D. Melgar and Rosemarie L. Gala, The Absorption and Biotransformation of Glyceryl Trinitrate-1,3-^{14}C by Rats, Biochem. Pharmacol., 17:2179-2183 (1968).

9. Needleman, P., D. J. Blehm, A. B. Harkey, E. M. Johnson, and S. Lang, The Metabolic Pathway in the Degradation of Glyceryl Trinitrate, J. Pharmacol. Exp. Ther., 179:347-353 (1971).

10. Hay, G. W , B. A. Lewis, and F. Smith, Thin-Layer Chromatography in the Study of Carbohydrates, J. Chromatog., 11:479-486 (1962).

PAPERS AND MANUSCRIPTS

A. Papers Presented to Scientific Meetings

Dilley, J. V., B. S. Anderson, D. N. Roberts, and C. C. Lee: The Acute Oral Toxicity of Several Munition Compounds and Their Synthetic Intermediates in Rodents. Fourth Annual Meeting for the Society of Toxicology, March 9-13, 1975, Williamsburg, Virginia. Abstract of Papers, 14:75, 1975.

Hodgson, J. R., L. M. Halfpap, and Cheng-Chun Lee: Absorption Distribution and Excretion of White Phosphorus in Rats. Fifty-nine Annual Meeting for the Federation of American Societies for Experimental Biology, April 13-18, 1975, Atlantic City, New Jersey. Fed. Proc., 34:74, 1975.

Cheng-Chun Lee, James V. Dilley, and John R. Hodgson: Toxicity and Disposition of 2,4-Dinitrotoluene. Sixth International Congress of Pharmacology, IUPHAR, July 20-25, 1975, Helsinki, Finland.

B. Manuscript Accepted for Publication

Hodgson, J. R., and C. C. Lee: Trinitroglycerol Metabolism: Denitration and Glucuronide Formation in the Rat. Toxicol. and Appl. Pharmacol.

C. Manuscripts in Preparation

Acute Toxicity of Nitrotoluenes, Nitroglycerins, Nitrocellulose, and White Phosphorus.

Disposition and Metabolism of Dinitroglycerols.

Disposition and Metabolism of Mononitroglycerols.

APPENDIX I

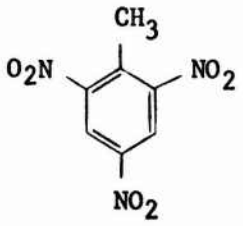
THE IDENTIFICATION AND ASSAY OF NITROTOLUENES AND NITROGLYCERINS

2,4,6-Trinitrotoluene
2,3-Dinitrotoluene
2,4-Dinitrotoluene
2,5-Dinitrotoluene
2,6-Dinitrotoluene
3,4-Dinitrotoluene
Trinitroglycerin
1,2-Dinitroglycerin
1,3-Dinitroglycerin
1-Mononitroglycerin
2-Mononitroglycerin

MIDWEST RESEARCH INSTITUTE
Contract No. DAMD17-74-C-4073
14 November 1974

DATA ON: 2,4,6-Trinitrotoluene .

SUBMITTED BY: James Dilley



Supplier: K & K Laboratories
Lot No.: 2,4,6-TNT-1

I. Identity

A. Capillary Melting Point

Observed 80-82°

Reported[1/] 81-82°

B. Spectra

1. Infrared: The IR spectrum (K Br wafer) of Lot 2,4,6-TNT-1 was compatible with the compound's structure and identical to the reported spectrum.[2/]

2. Nuclear Magnetic Resonance: The NMR of Lot 2,4,6-TNT-1 ($CDCl_3$) was compatible with the compound's structure and identical to the reported spectrum.[3/]

1/ Handbook of Chemistry and Physics, 44th Edition, pp. 1252-3.
2/ Sadtler Standard Spectra. Infrared No. 21886.
3/ Sadtler Standard Spectra. NMR No. 18215 M.

II. Assay

A. Elemental Analysis (calculated for $C_7H_5N_3O_6$)

Element	C	H	N
% Theoretical	37.01	2.22	18.50
% Observed	36.98	1.92	18.31

B. Thin-Layer Chromatography

1. Plates: Brinkmann Silica Gel NF

2. Solvent system: ethyl acetate/petroleum ether (15:85)

3. Material spotted:

100 µg	Lot 2,4,6-TNT-1	
10 µg	2,3-dinitrotoluene	
10 µg	2,4-	"
10 µg	2,5-	"
10 µg	2,6-	"
10 µg	3,4-	"
10 µg	3,5-	"

4. Detection: 5% diphenylamine in ethanol with 254 nm UV

5. Results: Lot 2,4,6-TNT-1 moved as a single spot with an R_f of 0.74.

III. Conclusions

Lot 2,4,6-TNT-1 (K & K Laboratories) contains 2,4,6-trinitrotoluene in a purity of ≥ 99%.

MIDWEST RESEARCH INSTITUTE

Nita West
Assistant Chemist

Sandra Reich
Assistant Chemist

M. Harris
Mike Harris
Assistant Chemist

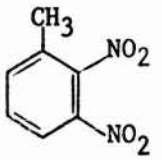
Approved:

Don Helton
Danny O. Helton
Associate Chemist

MIDWEST RESEARCH INSTITUTE
Contract No. DAMD17-74-C-4073
18 November 1974

Data on: 2,3-Dinitrotoluene

Submitted by: James Dilley



Supplier: K & K Laboratories
Lot No.: 2,3-DNT-1

I. Identity

A. Capillary Melting Point

Observed	60-61°
Reported[1/]	59.5-61.5°

B. Spectra

1. Infrared: The IR spectrum (KBr wafer) of Lot 2,3-DNT-1 was compatible with the compound's structure and identical to the reported spectrum.[1/]

2. Nuclear Magnetic Resonance: The NMR spectrum of Lot 2,3-DNT-1 (solvent $CDCl_3$) was compatible with the compound's structure and identical to the reported spectrum.[2/]

3. Ultraviolet: Lot 2,3-DNT-1 in methanol showed an absorption maximum at 255 nm in agreement with the reported spectrum.[3/]

1/ Sadtler Standard Spectra. Infrared No. 36399.
2/ Sadtler Standard Spectra. NMR No. 8072M.
3/ Sadtler Standard Spectra. UV No. 16335.

II. Assay

A. Elemental Analysis

Element	C	H	N
% calculated	46.11	3.30	15.37
% observed	46.37	3.55	15.37

B. Thin-Layer Chromatography

1. Plate - Brinkmann Silica Gel NF

2. Solvent system - ethyl acetate/petroleum ether (15:85)

3. Material spotted - 100 µg Lot 2,3-DNT-1
10 µg 2,4-dinitrotoluene
10 µg 2,5-dinitrotoluene
10 µg 2,6-dinitrotoluene
10 µg 3,4-dinitrotoluene
10 µg 3,5-dinitrotoluene

4. Detection - 5% diphenylamine in ethanol spray reagent

5. Results - The sample moved as a single spot with an R_f = 0.33.

C. Gas Chromatography

The sample was studied using the following system:

Gas chromatograph: Varian 200

Detector: Flame ionization

Column: 6' x 1/8", aluminum
1.5% DC LSX-3-0295
1.5% GE XE-60
on Gas Chrom Q

Injector T°: 150°

Detector T°: 200°

Column T°: 150

Flowrate: 40 cc N_2/min

No other dinitrotoluene derivatives were observed at the 1% concentration level.

III. Conclusions

The K & K Laboratory sample (Lot 2,3-DNT-1) contains 2,3-dinitrotoluene in a purity of > 99%.

MIDWEST RESEARCH INSTITUTE

Sandra Reich
Sandra Reich
Assistant Chemist

Nita West
Nita West
Assistant Chemist

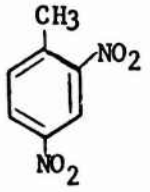
M. Harris
Mike Harris
Assistant Chemist

Approved:

Dan Helton
Danny O. Helton
Associate Chemist

MIDWEST RESEARCH INSTITUTE
Contract No. DAMD17-74-C-4073
18 November 1974

Data on: 2,4-Dinitrotoluene



Submitted by: James Dilley

Supplier: K & K Laboratories
Lot No.: 2,4-DNT-1

I. Identity

A. Capillary Melting Point

Observed - 70-72.5°
Reported[1] - 71°

B. Spectra

1. Infrared: The IR spectrum (KBr wafer) of the sample was compatible with the compound's structure and identical to the reported spectrum.[2]

2. Nuclear Magnetic Resonance: The NMR spectrum (solvent $CDCl_3$) of the sample was compatible with the compound's structure and identical to the reported spectrum.[3]

3. Ultraviolet: The sample in cyclohexane exhibited the same absorption maximum (233 nm) as in the reported spectrum.[4]

1/ Handbook of Chemistry and Physics, 50th edition, p. C-518.
2/ Sadtler Standard Spectra. Infrared No. 175.
3/ Sadtler Standard Spectra. NMR No. 3229.
4/ Sadtler Standard Spectra. UV No. 2550.

II. Assay

A. Elemental Analysis

Element	C	H	N
% calculated	46.11	3.30	15.37
% observed	46.36	3.32	15.32

B. Thin-Layer Chromatography

1. Plate: Brinkmann silica gel NF

2. Solvent System: ethyl acetate/petroleum ether (15:85)

3. Material Spotted: 100 µg Lot 2,4-DNT-1
10 µg 2,3-dinitrotoluene
10 µg 2,5-dinitrotoluene
10 µg 2,6-dinitrotoluene
10 µg 3,4-dinitrotoluene
10 µg 3,5-dinitrotoluene

4. Detection: 5% diphenylamine in ethanol spray

5. Results: Lot 2,4-DNT-1 moved as a single spot with a R_f = 0.52.

C. Gas Chromatography

The sample was studied using the following system:

Gas chromatograph: Varian 200

Detector: Flame ionization

Column: 6 ft x 1/8 in., aluminum
1.5% DC LSX-3-0295
1.5% GE XE-60
on Gas chrom Q

Injector T°: 150°

Column T°: 150°

Detector T°: 200°

Flow rate: 40 cc N_2/min

This work indicated 2,6-dinitrotoluene as an impurity in a concentration of 1.7%.

III. Conclusions

Lot 2,4-DNT-1 contains 98% 2,4-dinitrotoluene and about 2% 2,6-dinitrotoluene.

MIDWEST RESEARCH INSTITUTE

Sandra Reich
Sandra Reich
Assistant Chemist

Nita West
Nita West
Assistant Chemist

M. Harris
Mike Harris
Assistant Chemist

Approved:

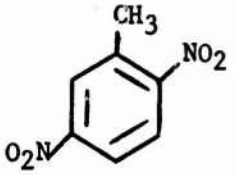
Don Helton

Danny O. Helton
Associate Chemist

MIDWEST RESEARCH INSTITUTE
CONTRACT NO. DAMD17-74-C-4073
6 March 1975

Data On: 2,5-dinitrotoluene

Submitted by: James Dilley



Supplier: K & K Laboratories
Lot No.: 2,5-DNT-1

I. Identity

A. Capillary Melting Point

Observed 48-51°

Reported 50.5-52.5°

B. Spectra

1. Infrared

The IR spectrum (KBr wafer) was compatible with the structure and identical to the reported spectrum.[2]

2. Nuclear Magnetic Resonance

The NMR spectrum was determined in $CDCl_3$ using a Varian A-60 spectrometer. The spectrum was compatible with the structure and similar to that reported.[3] However, the presence of some minor peaks suggested the presence of more than one dinitrotoluene isomer.

3. Ultraviolet Spectrum

The sample exhibited a maximum at 258 nm in 95% ethanol with an ϵ value of 11,200. The literature reports a λmax of 258 nm.[2]

1/ Handbook of Chemistry and Physics, 41st Edition, p. 1248.
2/ Varsanyi, G., S. Holly, and L. Fenichel, Acta.
3/ Mathias, A., and D. Taylor, Analytical Chimica Acta, 35, 376 (1966).

II. Assay

A. Elemental Analysis

Element	C	H	N
% Observed 4/	45.95	3.05	15.11
% Theoretical	46.11	3.30	15.37

B. Thin-Layer Chromatography

1. Plate: Brinkmann Silica Gel F

2. Solvent system: Ethyl Acetate/Petroleum Ether (15/85)

3. Material spotted: 200 µg Lot 2,5-DNT-1
2,4,6,10,20 µg 2,6-DNT
2,4,6 µg 2,3-DNT

4. Detection: UV (254 nm)

5. Results: Lot 2,5-DNT-1 showed a major spot at R_f=0.7 and two minor spots at R_f=0.5 and 0.3. The spot at R_f=0.5 is probably 2,6-DNT in a concentration of 2 to 3%. The spot of R_f=0.3 may be 2,3-DNT in a concentration of ≤ 1%.

C. Gas Chromatography

The sample was studied using the following system:

Gas chromatograph: Varian 200

Detector: Flame ionization

Column: 6 ft x 1/8 in
1.5% DC-LSX-3-0295
1.5% GE XE-60
on Gas chrom Q

Injector T°: 150°

Detector T°: 200°

Column T°: 150°

4/ Assayed by MRI.

Flowrate: 40 cc/min

The 2,6-dinitrotoluene isomer is present in a concentration of 4 ± 1%. The 2,3-dinitrotoluene isomer is present in a concentration of 1%.

III. Conclusions

The K & K Laboratory sample (Lot 2,5-DNT-1) contains 2,3-dinitrotoluene in a purity of 95 ± 1% plus 4 ± 1% 2,6-dinitrotoluene and 1% 2,3-dinitrotoluene.

MIDWEST RESEARCH INSTITUTE

S.R./D.H.

Sandra Reich
Assistant Chemist

N.W/D.H.

Nita West
Assistant Chemist

M.H./D.H.

Mike Harris
Assistant Chemist

Approved:

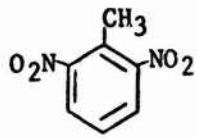
Don Helton

Danny O. Helton
Associate Chemist

MIDWEST RESEARCH INSTITUTE
Contract No. DAMD17-74-C-4073
4 November 1974

Data on: 2,6-Dinitrotoluene

Submitted by: James Dilley



Supplier: K & K Laboratories, Inc.
Lot: 2,6-DNT-1

I. Identity

A. Capillary Melting Point

Observed	58-61°
Reported[1]	66°

B. Spectra

1. Infrared: The IR spectrum of the sample (KBr pellet) was compatible with the compound's structure and identical to the reported spectrum.[2]

2. Nuclear magnetic resonance: The NMR spectrum of the sample was determined in $CDCl_3$ using a Varian A-60 spectrometer. The spectrum was identical to that reported.[3]

II. Assay

A. Elemental Analysis

Element	C	H	N
% Calculated	46.11	3.30	15.37
% Observed[4]	45.82	3.57	15.12

1/ Handbook of Chemistry and Physics, 50th edition, p. C-518.
2/ Sadtler Standard Spectra, Infrared No. 17,378.
3/ Sadtler Standard Spectra, NMR No. 895.
4/ Analyzed by MRI.

B. Thin-Layer Chromatography

1. Plate: Brinkmann Silica Gel F
Brinkmann Silica Gel NF

2. Solvent system: Ethyl acetate/petroleum ether (15:85)

3. Amount spotted: 100 µg K&K 2,6-dinitrotoluene
1, 2, 5, 10 µg 2,4-dinitrotoluene

4. Detection: 5% diphenylamine in ethanol spray, or UV (254 nm)

5. Results: Using Silica Gel NF with 5% diphenylamine for detection, a single spot with an R_f = 0.6 was observed. Using Silica Gel F with UV (254 nm) for detection, the major spot had an R_f = 0.6, while a minor spot (R_f = 0.7) with the R_f of 2,4-DNT was observed. The concentration of the impurity was < 1%.

C. Gas Chromatography

The sample was studied using the following system:

Gas chromatograph: Varian 200

Detector: Flame ionization

Column: 6' x 1/8", aluminum
1.5% DC LSX-3-0295
1.5% GE-XE-60
on gas chromatography Q

Injector T°: 150°

Column T°: 150°

Detector T°: 200°

Flow rate: 40 CC N_2

This work also indicated that the concentration of the 2,4-DNT impurity was < 1%.

III. Conclusions

The K & K Laboratory sample of 2,6-dinitrotoluene is ≥ 99%.

MIDWEST RESEARCH INSTITUTE

Sandra Reich

Sandra Reich
Assistant Chemist

Nita West

Nita West
Assistant Chemist

M. Harris

Mike Harris
Assistant Chemist

Approved:

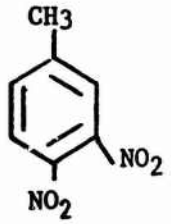
Dan Helton

Danny O. Helton
Associate Chemist

MIDWEST RESEARCH INSTITUTE
Contract No. DAMD17-74-C-4073
6 November 1974

Data on: 3,4-Dinitrotoluene

Submitted by: James Dilley



Supplier: K&K Laboratories
Lot No.: 3,4-DNT-1

I. Identity:

A. Capillary Melting Point

Observed 57-61°

Reported[1] 59-61°

B. Spectra:

1. Infrared: The IR spectrum (K Br wafer) of Lot 3,4-DNT-1 was compatible with the structure and identical to the reported spectrum[2]

2. Nuclear Magnetic Resonance: The NMR spectrum of Lot 3,4-DNT-1 was determined in $CDCl_3$ on a Varian A-60 spectrometer. The spectrum was identical to that reported.[3]

3. Ultraviolet: In methanol Lot 3,4-DNT-1 shows λ_{max} at 262 nm (ϵ = 6200) and 217 nm (ϵ = 13,000). The literature reports λ_{max} at 262 nm (ϵ = 5900) and 217 nm (ϵ = 12,400).[4]

1/ Handbook of Chemistry and Physics, 44th Edition, p 1250
2/ Sadtler Spectral Index. Infrared No. 1949.
3/ Sadtler Spectral Index. NMR No. 8090M.
4/ Sadtler Spectral Index. UV No. 15815.

II. Assay

A. Elemental Analysis

Element	C	H	N
%Observed[5/]	46.03	3.14	15.20
%Theory	46.11	3.30	15.37

B. Thin-Layer Chromatography

1. Plate: Brinkmann Silica Gel NF

2. Solvent System: ethyl acetate/petroleum ether (15:85)

3. Material spotted: 100 µg Lot 3,4-DNT-1
50 µg 2,4-DNT
50 µg 2,5-DNT
50 µg 2,6-DNT
50 µg 3,5-DNT

4. Results: Lot 3,4-DNT-1 traveled as a single spot with an $R_f = 0.33$.

C. Gas Chromatography

Lot 3,4-DNT-1 was studied using the following system:

Gas Chromatograph: Bendix 2500

Column: 6' x 1/8" I.D.
1.5% GE-XE-60
1.5% DC LSX-3-0295
on Gas chrom Q

Injection Temperature: 135°

Column Temperature: 135°

Nitrogen Flow: 30 cc/min

Detector Temperature: 200°

No other dinitrotoluene toluene isomers were detected.

III. Conclusions

Lot 3,4-DNT-1 (K&K Laboratories) contains 3,4-dinitrotoluene in a purity of ≥99%.

MIDWEST RESEARCH INSTITUTE

Anita West
Assistant Chemist

Sandra Reich
Assistant Chemist

Mike Harris
Assistant Chemist

Approved:

Danny O. Helton
Associate Chemist

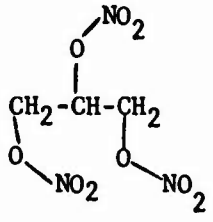
5/ Assayed by MRI.

MIDWEST RESEARCH INSTITUTE
CONTRACT NO. DAMD-17-74-C-4073
7 July 1975

Data on: Trinitroglycerin

Supplier: Atlas Chemical Division
of ICI America Inc.

Lot No.: D17-H3



10% trinitroglycerin on lactose

I. Identity

The sample was extracted with chloroform to remove the trinitroglycerin. Evaporation of the chloroform gave a liquid whose infrared spectrum (between salt plates) was identical to that reported for trinitroglycerin.[1/]

II. Assay

A. Gas Chromatography

The sample was studied by gas chromatography using the following system:

1. Instrument: Bendix 2500 equipped with flame ionization detector
2. Column: glass, 6 ft x 1/4 in
   1.5% DC LSX-3-0295
   1.5% GE-XE-60
   on Gas Chrom Q
3. Nitrogen flow: 30 cc/min
4. Detector T°: 200°
5. Injector T°: 130°
6. Column T°: 130°

1/ Alma L. Hayden, Oscar R. Sammul, George B. Selzer, and Jonas Carol, "Infrared and Ultraviolet Spectra of Some Compounds of Pharmaceutical Interest," Association of Offical Analytical Chemists, Washington, 1972, p. 150.

7. References: 1-mononitroglycerin, 2-mononitroglycerin, 1,2-dinitroglycerin, 1,3-dinitroglycerin, U.S.P. trinitroglycerin

8. Results: With the peak for trinitroglycerin representing 100%, no other peaks were observed at 1% concentration level by peak area comparison.

B. Nitro Content

The Lot was analyzed for nitroglycerin content by the method of Wells.[2/] The Lot contains 9.72 ± 0.09% trinitroglycerin.

III. Conclusions

Lot D17-H3 contains 9.72 ± 0.09% trinitroglycerin.

MIDWEST RESEARCH INSTITUTE

John Rollheiser

John Rollheiser
Junior Chemist

Approved:

Dan Helton

Danny O. Helton
Associate Chemist

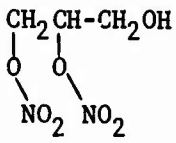
2/ Clyde E. Wells, Harvey M. Miller, and Yvonne H. Pfabe, Journal of the Association of Official Analytical Chemists, 53, 579 (1970).

MIDWEST RESEARCH INSTITUTE
Contract No. DAMD17-74-C-4073
2 July 1975

Data on: 1,2-Dinitroglycerin

Supplier: Midwest Research Institute

Lot: 1,2-DNG-1



I. Identity

A. Infrared Spectrum

The IR spectrum of the neat sample (between KBr plates) was identical to that of the reported spectrum.[1/]

B. Benzoate Derivative

The 3,5-dinitrobenzoate derivative when crystallized from ethyl ether melted at 86.5 to 88°. The reported melting point is 88°.[2/]

II. Assay

A. Gas Chromatography

The sample was studied using the following system:

1. Instrument: Bendix 2500 equipped with flame ionization detector.

2. Column: glass, 6 ft x 1/2 in. I.D.
1.5% DC LSX-3-0295

1/ Dr. Robert J. Baczuk (Hercules Incorporated, Magna, Utah) provided a spectrum of the 1,2-dinitroglycerol synthesized by I. Dunstan, J. V. Griffiths and S. A. Harvey, J. Chem. Soc., p. 1319 (1965).

2/ Dunstan, I., J. V. Griffiths, and S. A. Harvey, "Nitric Esters. Part I. Characterisation of the Isomeric Glycerol Dinitrates," J. Chem. Soc., p. 1319 (1965).

1.5% GE-XE-60
on Gas Chrom Q

3. Nitrogen flow: 30 cc/min.

4. Detector T°: 200°

5. Injector T°: 130°

6. Column T°: 130°

With the major peak representing 100%, no other peaks were observed at the 1% concentration level by comparison of peak areas.

B. Thin-Layer Chromatography

1. Plate: Brinkmann Silica Gel F-254

2. Solvent system: hexane/ethyl acetate (2:1)

3. Material spotted: 100 µg Lot 1,2-DNG-1

10 µg 1,3-dinitroglycerin

10 µg 1-mononitroglycerin

10 µg 2-mononitroglycerin

4. Detection: UV (254 nm), I_2 vapor

5. Results: Lot 1,2-DNG-1 gave a single spot.

C. Nitro Group Concentration

The solvent (hexane/ethyl acetate) was not removed from the bulk sample due to the explosion hazard. The concentration of 1,2-DNG was determined using the nitro group determination method of Selig.[3/] Using this assay method, the bulk sample was diluted to a concentration of 50% 1,2-DNG.

3/ Selig, Walter, "Microdetermination of Aromatic Nitro Compounds, Nitrocellulose, and Cyclic Nitramines," AEC Report UCRL 3369.

III. Conclusions

Lot 1,2-DNG-1 contains 1,2-dinitroglycerin as the only major organic nitrate component in a concentration of 50% dissolved in hexane/ethyl acetate.

MIDWEST RESEARCH INSTITUTE

M.H./D.H.

Mike Harris
Assistant Chemist

David Ebert

Dave Ebert
Assistant Chemist

Approved:

Don Helton

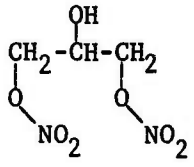
Danny O. Helton
Associate Chemist

MIDWEST RESEARCH INSTITUTE
Contract No. DAMD-17-74-C-4073
2 July 1975

Data on: 1,3-Dinitroglycerin

Supplier: Midwest Research Institute

Lot: 1,3-DNG-1



I. Identity

A. Infrared Spectrum

The IR spectrum of the neat sample (between KBr plates) was identical to that of the reported spectrum.[1/]

B. Benzoate Derivative

The benzoate derivative when crystallized from methanol melted at 65 to 66°. The reported melting point is 66°.[2/]

II. Assay

A. Gas Chromatography

The sample was studied by gas chromatography using the following system:

Instrument: Bendix 2500 equipped with flame ionization detector.

Column: glass, 6 ft x 1/8 in. I.D.
1.5% DC LSX-3-0295

1/ Dr. Robert J. Baczuk (Hercules Incorporated, Magna, Utah) provided a spectrum of the 1,3-dinitroglycerol synthesized by I. Dunstan, J. V. Griffiths and S. A. Harvey, J. Chem. Soc., p. 1319 (1965). The spectrum was not published.

2/ Dunstan, I., J. V. Griffiths, and S. A. Harvey, "Nitric Esters. Part I. Characterisation of the Isomeric Glycerol Dinitrates," J. Chem. Soc., p. 1319 (1965).

1.5% GE-XE-60
on Gas Chrom Q

Nitrogen Flow: 30 cc/min

Detector T°: 200°

Injector T°: 130°

Column T°: 130°

With the major peak representing 100%, no other peaks were observed at the 1% concentration level by comparison of peak areas.

B. Thin-Layer Chromatography

1. Plate-Brinkmann Silica Gel F-254

2. Solvent system: hexane/ethyl acetate (2:1)

3. Material spotted: 100 µg Lot 1,3-DNG-1

10 µg 1,2-dinitroglycerin

10 µg 1-mononitroglycerin

10 µg 2-mononitroglycerin

4. Detection: UV (254 nm), I_2 vapor

5. Results: Lot 1,3-DNG-1 gave a single spot.

C. Nitro Group Concentration

The solvent (hexane/ethyl acetate) was not removed from the bulk sample due to the explosion hazard. The concentration of 1,3-dinitroglycerin was determined using the nitro group determination method of Selig.[3/] Using this assay method, the bulk sample was diluted to a concentration of 50% 1,3-dinitroglycerin.

3/ Selig, Walter, "Microdetermination of Aromatic Nitro Compounds, Nitrocellulose, and Cyclic Nitramines," AEC Report UCRL 3369.

III. Conclusions

Lot 1,3-DNG-1 contains 1,3-dinitroglycerin as the only major organic nitrate component in a concentration of 50% dissolved in hexane/ethyl acetate.

MIDWEST RESEARCH INSTITUTE

M.H./D.H.

Mike Harris
Assistant Chemist

Dave Ebert

Dave Ebert
Assistant Chemist

D.A./D.H.

James Althaus
Senior Chemist

Approved:

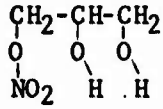
Dan Helton

Danny O. Helton
Associate Chemist

MIDWEST RESEARCH INSTITUTE
CONTRACT NO. DAMD17-74-C-4073
20 December 1974

Data on: 1-mononitroglycerin (1-MNG)

Submitted by: James Dilley



Supplier: Midwest Research Institute
Lot No.: 1-MNG-11/1/74

I. Identity

A. Infrared Spectrum

The spectrum (KBr wafer) was compatible with the structure and similar to a reference spectrum supplied by the U.S. Naval Ordnance Laboratory.[1/]

B. Capillary Melting Point

Observed	57-58°
Reported[2/]	58°

C. Dibenzoate Derivative

The lot was reacted with benzoyl chloride to prepare the dibenzoate derivative.

Observed melting point of Dibenzoate[3/]	67.5-70.5°
Melting point reported[4/]	68°

1/ Elecnore G. Kayser, U.S. Naval Ordnance Laboratory, White Oak, Silver Spring, Maryland 20910.

2/ Handbook of Chemistry and Physics, 41st edition, pp. 1,012-1,013.

3/ Observed by differential scanning colorimetry.

4/ I. Dunstan, J. V. Griffiths, and S. A. Harvey, J. Chem. Soc., p. 1,325, 1965.

D. Nuclear Magnetic Resonance

The Lot was studied in CD_3OD, $CDCl_3$, and CD_3COCD_3 using a Varian A-60. The absorption multiplicity was solvent and temperature dependent. The absorption range was 3.5 to 4.7 δ. This data is consistent with the structure.

II. Assay

A. Elemental Analysis (calculated as $C_3H_7NO_5$)

Element	C	H	N
% observed	26.51	5.45	9.96
% calculated	26.28	5.15	10.22

B. Thin-Layer Chromatography

1. Plate - Brinkmann aluminum oxide F

2. Solvent system - methanol/chloroform (20/80)

3. Material spotted - 10, 100, 200, 300, 500 µg Lot 1-MNG-11/1/74
200, 500 µg 2-mononitroglycerin[5/]

4. Detection - UV (254 nm), I_2 vapor

5. Results - A single spot (R_f = 0.5) was observed.
The 2-mononitroglycerol has an R_f = 0.7.

C. Gas Chromatography

Lot 1-MNG-11/1/74 was studied using the following system:

Instrument: Bendix 2500

Column: glass, 6 ft x 1/8 in.
1.5% DC LSX-3-0295
1.5% GE XE-60
on Gas Chrom Q

Flow rate: 30 cc N_2/min.

5/ Supplied by Dan Helton, Midwest Research Institute.

Detector T°: 200°

Injector T°: 130°

Column T°: 130°

The lot gave a single peak at approximately 4 min. retention time. Isomeric 2-mononitroglycerine has a retention time of 1.60 relative 1.00 for 1-mononitroglycerin.

III. Conclusions

Lot 1-MNG-11/1/74 contains 1-mononitroglycerin in a purity of > 99%.

MIDWEST RESEARCH INSTITUTE

N West

Nita West
Assistant Chemist

M. Harris

Mike Harris
Assistant Chemist

Approved:

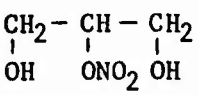
Don Helton

Danny O. Helton
Associate Chemist

MIDWEST RESEARCH INSTITUTE
Contract No. DAMD17-74-C-4073
20 December 1974

Data on: 2-Mononitroglycerin

Submitted by: James Dilley



Supplier: Midwest Research Institute
Lot No.: 2-MNG-11/18/74

I. Identity

A. Infrared Spectrum

The spectrum (KBr wafer) was consistent with the structure and similar to a reference spectrum.[1/]

B. Capillary Melting Point

Observed 54-55°

Reported[2/] 54°

C. Dibenzoate Derivative

Lot 2-MNG-11/18/74 was reacted with benzoyl chloride to prepare the dibenzoate derivative.

Melting point of dibenzoate 56-59°

Reported melting point 56°[3/]

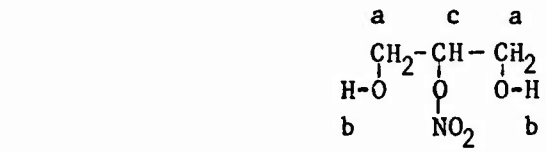
1/ The reference spectrum was provided by Eleonore G. Kayser, U.S. Naval Ordnance Laboratory, White Oak, Silver Spring, Maryland 20910.

2/ Handbook of Chemistry and Physics, the Chemical Rubber Company, 50th Edition, p. C-313.

3/ Dunstan, I., J. V. Griffiths, and S. A. Harvey, J. Chem. Soc., 1325, 1965.

D. Nuclear Magnetic Resonance

The spectrum was obtained in CD_3OD using a Varian A-60 instrument. The following spectrum was observed:



Proton Group	Comment
a	3.86 δ, doublet, J=5.0
b	4.53 δ, broad singlet
c	5.23 δ, pentet, J=5.0

III. Assay

A. Elemental Analysis

Element	C	H	N
% Calculated	26.28	5.15	10.22
% Observed[4/]	26.46	5.28	9.90

B. Gas Chromatography

The sample was studied using the following system:

Instrument: Bendix 2500

Column: glass, 6 ft x 1/8 in. I.D.
1.5% DC LSX-3-0295
1.5% GE-XE-60
on Gas Chrom Q

Nitrogen flow: 30 cc/min

4/ Assayed by Midwest Research Institute.

Detector T°: 200°

Injector T°: 130°

Column T°: 130°

The 2-mononitroglycerin has a retention time of 1.6 relative to 1.0 for 1-mononitroglycerin.[5/] Lot 2-MNG-11/18/74 contains 1.5% 1-mononitroglycerin by GC assay.

III. Conclusions

Lot 2-MNG-11/18/74 contains 2-mononitroglycerin in a purity of 98.5% and 1.5% 1-mononitroglycerin.

MIDWEST RESEARCH INSTITUTE

N West
Nita West
Assistant Chemist

Sandra Reich
Sandra Reich
Assistant Chemist

M. Harris
Mike Harris
Assistant Chemist

Approved:

Dan Helton

Danny O. Helton
Associate Chemist

5/ Supplied by Midwest Research Institute.